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13. ABSTRACT (Maximum 200 Words) During the first year of our Institutional Breast Cancer Research Training Program at the FCCC we have recruited, from a pool of 26 applicants, four trainees (Drs. Lareef, Sherman, Zalatoris and Pan). Four main areas of research are in progress. Dr Lareef is studying the functional role of chromosomes 11 and 17 in the process of immortalization and transformation of human breast epithelial cells. Transfer of chromosome 17 in to transformed human breast epithelial cells (BP1-E) reverts the transformation phenotypes and the Fas mediated apoptosis. The gene or genes that are controlling this process are located in the chromosome 17p13.1-13.2(D17S796). Dr. Sherman has initiated the study on lymphedema prevention in breast cancer survivors. Dr. Zalatoris is studying the polymorphisms of human UGT1A6 and UGT1A9 genes and functional differences of the variant gene products. Dr. Pan is studying the role of γ -synuclein in breast cancer progression and metastasis. He has identified ERK1/2 and JNK as protein kinases interacting with γ -synuclein and determining that the interaction between ERK1/2 and γ -synuclein is independent of the activation status of ERK1/2. Lastly he has shown that stress-induced (i.e., UV radiation, heat shock, and sodium arsenite) JNK activation can be specifically blocked by γ -synuclein.				
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A-INTRODUCTION

The focus of the FCCC Institutional Breast Cancer Training Program (IBCTP) is to integrate the unique talents and interests of the Center's basic scientists, clinical investigators and behavioral scientists to create a comprehensive effort to approach the problems of breast cancer. The rich scientific and intellectual environment of FCCC is nurtured by a cohesive interdisciplinary program that is based on expertise in areas of high relevance to breast cancer. The Institutional Breast Cancer Training Program offers to the postdoctoral trainees practical experience in the fields of cellular and molecular biology, drug resistance and targeted immunotherapy, genetic epidemiology and control, psychosocial and behavioral medicine, as well as breast cancer prevention, diagnosis, and treatment.

B-BODY

B-i. Organization of the FCCC-Institutional Breast Cancer Training Program. Following our statement of work we have accomplished the following tasks:

Task 1. During the first three months of our program we have established a *Recruitment Panel* formed by our Faculty members and developed a web page <http://www.fccc.edu/postdoc/BreastCaTraining.html>, advertised in national meeting, such as the American Association for Cancer Research in which a poster and request information brochures were distributed (Appendix, Exhibit A), placed an advertisement in Science (Appendix , Exhibit B), and contacted scientists in the breast cancer area by phone and e-mail.

Task 2. The *Recruitment Panel* and the *Applications Evaluation Committee* are formed by members of the Faculty and the Advisory Panel, that were established at the time of the application was written. All the applicants were required to present a statement of the fellow's background, training, and professional interests and goals, and a minimum of three recommendation letters. Fellows applying to the program must have a Ph.D. or M.D. degree with background in biology, molecular biology; chemistry, including organic and physical chemistry; mathematics; biochemistry; genetics, and or behavioral sciences. Important evaluation criteria used by *The Recruitment Panel* and the *Applications Evaluation Committee* was the evaluation of personal statements of research and career goals, previous laboratory research experience, publications, and the recommendation letters submitted.

As a result of our **task 1**, we received 10 applications that were evaluated as described above and three candidates were chosen: Dr Hasan, M. Lareef, Dr. Sarah Fashema and Dr. Kerry Sherman. Dr Lareef started working in the middle of July 2000, in a project that is described in section **B-ii** of this report. Dr Fashema started working also by the middle of July 2000, but she resigned after three months because of geographic relocation (see report in **section B-iii**). Dr Sherman joined us in December of 2000, (see report in **section B-iv**). In order to fill the vacant spots, *the Recruitment Panel* and the *Applications Evaluation Committee* decided to run another set of advertisement similar to the one run in **Task 1**. At the beginning of December 2000 we have received 16 applications, that were evaluated as indicated above, and two candidates were selected, Dr Zalatoris and Dr. Pan whose reports are described in sections **B-v** and **B-vi** respectively. Dr Zalatoris joined the program in the middle of December and Dr Pan in January of 2001.

Task 3. Postdoctoral training. The training was organized in sixteen modules as has been described in the original application. Module V directed by Dr Kent W Hunter was changed due to the departure of Dr Hunter from the FCCC. The Faculty and the Advisory Panel of the Institutional Breast Cancer Training Program suggested that Dr Rebecca Raftogianis must be in charged of Module V now named Genomic Polymorphism (See in Appendix as Exhibit C, the biosketch of Dr Raftogianis). The trainees have

already selected their modules at the time of the admission to the program. All of them have rotated in a two weeks period in the Module XV, Library Instruction and Training, and only one Dr Lareef have rotated in addition to the Module XV in Module XIV, Biostatistic. All the trainees must attend a special set of seminars to increase their general level of knowledge in the breast cancer field (See Appendix, Exhibit D). In addition the trainees must attend at least one general lecture a week from those offered by the Fox Chase Cancer Center. We have established a half-day seminar twice a year in which the trainees present their work in front of the Faculty and the Advisory Panel. The first one took place in March of 2001 and the second one has been scheduled for September of this year.

B-ii- Study of the functional role of chromosomes 11, and 17 in the process of immortalization and transformation of human breast epithelial cells.

Trainee: Hasan, M. Lareef, M.D.
Mentor: Jose Russo, MD
Period reported: July 30, 2000 – to July 30, 2001

Introduction. Breast Cancer is the most common malignancy in women and one third of all new cases of female's cancer in North America. The cause or causes of Breast Cancer initiation is not known. However, it has been found that in primary breast cancer genomic alterations play major role such as oncogene activation, loss of genetic material (LOH) and alterations in mismatch repair genes in the initiation and progression of the disease (1-4). Up to now loss of genetic material has been identified in at least 11 different chromosomes. The functionality of genomic alterations has been demonstrated by utilizing microcell-mediated chromosome transfer (MMCT), a technique that has been widely used for analyzing the functional role-played by gene/s located on a candidate chromosome (5-8). This technique has allowed scientists to map putative tumor suppressor genes (9-12), senescence genes (13) and metastasis genes (14, 15) to various chromosomes in human cancer cells. To further understand and identify which specific gene/s are involved in the process of transformation and programmed cell death we have developed an *in vitro* experimental system in which a spontaneously immortalized human breast epithelial cell (HBEC) line, MCF10F, has been transformed with chemical carcinogen (16-18). MCF10-F cells, treated with the carcinogen Benz (a) Pyrene (BP) originated the BP1-E cells. These cells express *in vitro* phenotypes indicative of neoplastic transformation, such as advantageous growth, anchorage independence, enhanced chemo invasiveness, loss of ductulogenic capacity in collagen and resistant to Fas mediated apoptosis as well as tumorigenic capacity in a heterologous host *in vivo* (17, 18). This *in vitro-in vivo* system has served as a useful model for studying the molecular and genetic mechanisms that may be involved in the initiation and progression of human breast cancer. In our laboratory, we have demonstrated that, allelic loss (LOH) and micro satellite instability (MSI) occurs in Chromosomes 11 and 17 in chemically transformed cells, in loci similar to those reported in breast cancer (19-24). In order to determine the functional role of these genomic changes we have used microcell mediated chromosome transfer (MMCT) to demonstrate if the restitution of fragment (s) of the chromosomes 11 and 17 could abrogate or aggravate the transformation phenotypes as well as resistant to Fas mediated apoptosis of these chemically transformed cells.

BODY.

Transformation phenotypes. Following transferring of chromosome 11 and 17 by MMCT we generated 16 and 18 clones respectively. We randomly selected 10 clones for each chromosome (BP1-E-11*neo* and BP1E-17*neo*) for further genotyping and phenotyping studies. MCF10F cells grew forming a monolayer of polyhedral cells without overlapping as previously described. BP1-E cells exhibited a growth pattern similar to MCF10-F cells, although the cells show a tendency to overlap. Seven out of 10 clones of BP1-E-17*neo* showed a morphology and population doubling similar to immortal MCF10-F cells, whereas the other three clones have the same morphology of the BP1-E cells. All the ten clones of BP1-E-11*neo* have the same morphological pattern than the parental BP1-E cells. MCF 10F cells do not form colonies in agar methocel (Figure 1A) instead BP1-E cells grew in agar-methocel and produce abundant number of colonies (Figure 1B). The seven clones of BP1-E-17*neo* that did not form colonies persisted as single cells or very few smaller colonies less than 50 μ m after 21 days (Figures 1C, 1E, 1H, 1I, 1J, 1K, 1L). The other three clones with chromosome 17 transfected grew in agar-methocel and produce numerous colonies like the parental BP1-E cells (Figures 1D, 1F and 1G). All the clones derived from BP1-E 11*neo* cells grew in agar-methocel and produced colonies (Figures. 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L). In

figure 3a and 3b are depicted the colony efficiency for MCF10F, BP1-E, BP1-E17neo and BP1-E-11neo cells. The differences observed are statistically significant.

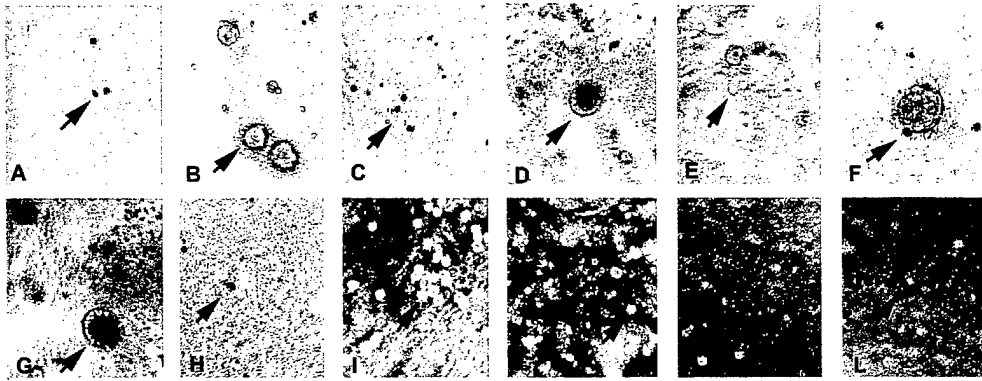


Figure 1: Cells plated in agar-methocel for colony assay after 21 days. MCF10F (A, 10X) no colonies and only isolated single cells, BP1-E(B 10X) colonies, BP1-E-17 neo I#1(C,10X)no colonies and only isolated cells, BP1-E 17 neo II# 2 (D, 10X) colonies, BP1-E 17 neo II# 3 (E, 10X) no colonies and only isolated cells, BP1-E 17 neo III# 1(F, 10X) colonies, BP1-E 17 neo III# 4(G, 10X) colonies, BP1-E 17 neo IV# 2(H, 10X) no colonies and only isolated cells, BP1-E 17 neo VI# 1 (I, 10X) no colonies and only isolated cells, BP1-E 17 neo VI# 2(J, 10X) no colonies and only isolated cells BP1-E 17 neo VI# 3 (K, 10X) no colonies and only isolated cells and BP1-E 17 neo VI# 4 (E, 10X) no colonies and only isolated cells.

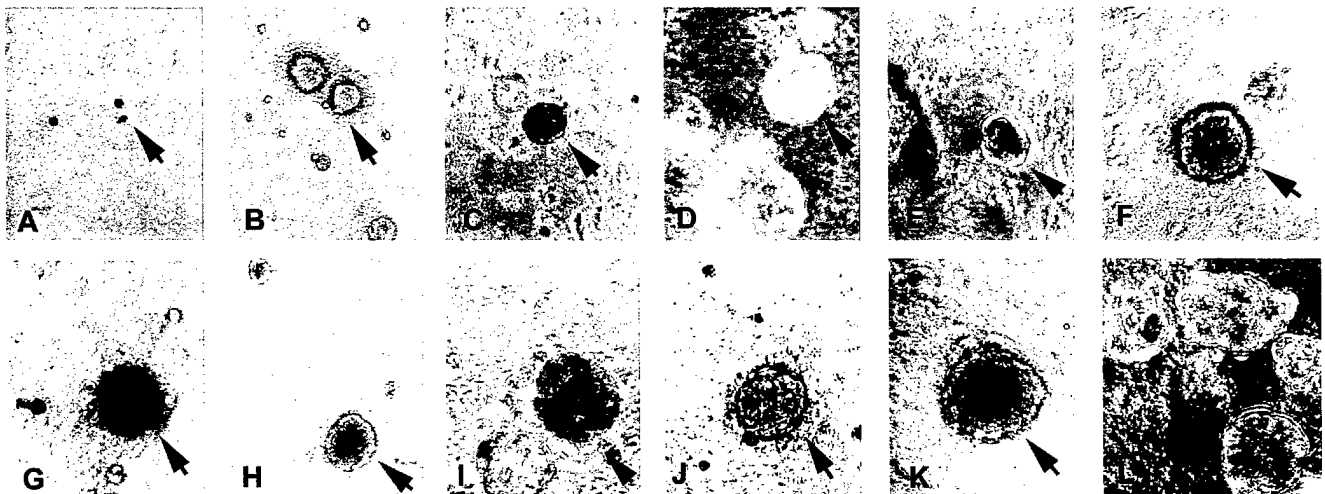


Figure 2: Chromosome 11 transfected cell lines in agar-methocel 21 days. MCF10F (A, 10X) no colonies and only isolated single cells, BP1-E (B, 10X) colonies, BP1-E 11 neo I 1# 1(C, 10X) colonies, BP1-E 11 neo I 1# 2(D 10X) colonies, BP1-E 11 neo I 1# 3 (E, 10X) colonies, BP1-E 11 neo I 1# 4(F, 10X) colonies, BP1-E 11 neo I 2# 1(G, 10X) colonies, BP1-E 11 neo I 2# 2(H, 10X) colonies, BP1-E 11 neo I 3# 1(I, 10X) colonies, BP1-E 11 neo I 3# 2(J, 10X) colonies, BP1-E 11 neo I 3# 3(K, 10X) colonies, BP1-E 11 neo I 3# 4(L, 10X) colonies,

MCF10-F cells grew in collagen matrix forming ductule like structures after 2-3 weeks (Figure 4A), whereas the BP1-E cells have lost the ductulogenic capacity and formed ball like structures in 3 weeks (Figure 4B). The same clones of BP1-17neo cells that did not form colonies in agar methocel formed ductules in collagen similar to those of the MCF10F cells (Figures 4 C, 4E, 4H, 4I, 4J, 4K, 4L). The clone of cells of BP1-E-17neo and BP1-E11neo that did formed colonies in agar methocel were unable to form ductules in collagen matrix and formed solid masses as depicted in figures Fig. 5C, 5D, 5E, 5F, 5G, 5H, 5I, 5J, 5K, 5L.

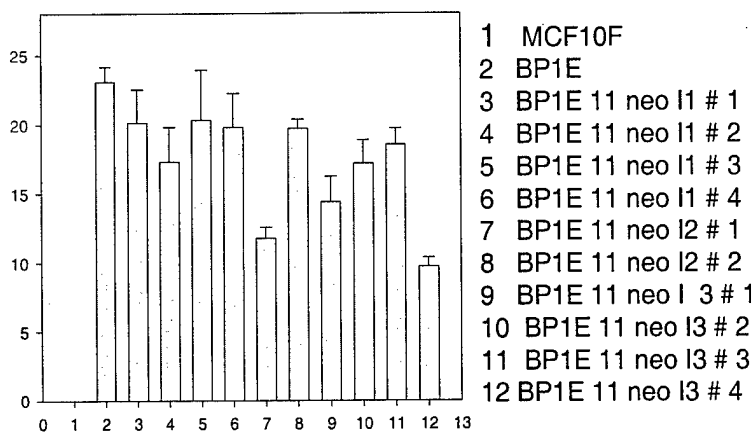


Figure 3A: Colony efficiency, of chromosome 11 transfected clones.

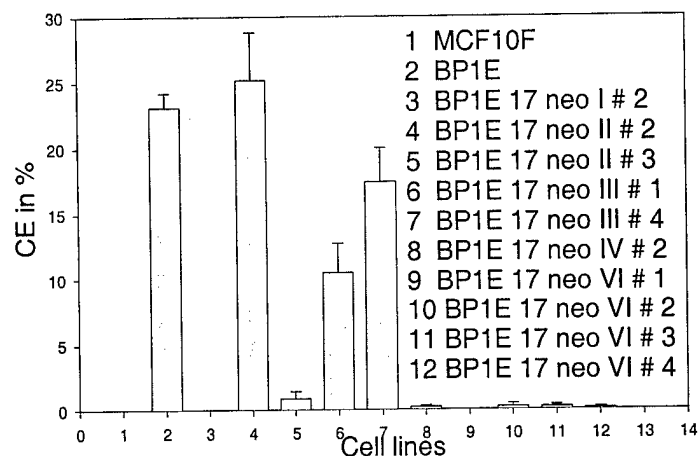


Figure 3B: Colony efficiency, of chromosome 17 transfected clones.

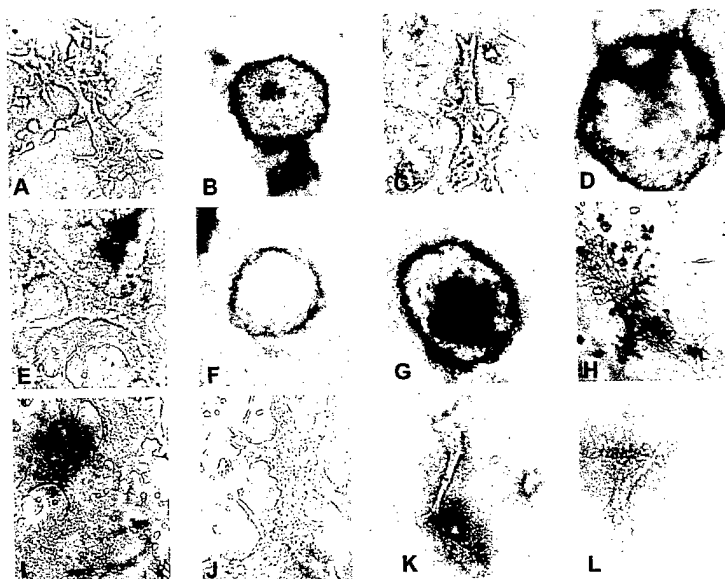


Figure 4: Ductulogenic capacity of chromosome 17 transfected clones in collagen: MCF10F (A, 10X) forms ducts, BP1-E (B 10X) ball like structure, BP1E-17 neo I#1(C, 10X) ducts, BP1-E 17 neo II# 2 (D, 10X) ball like masses, BP1-E 17 neo II# 3 (E, 10X) ducts, BP1-E 17 neo III# 1(F, 10X) ball like masses, BP1-E 17 neo III# 4(G, 10X) ball like masses, BP1-E 17 neo IV# 2 (H, 10X) ducts, BP1-E 17 neo VI# 1 (I, 10X) ducts, BP1-E 17 neo VI# 2 (J, 10X) ducts BP1-E 17 neo VI# 3 (K, 10X) ducts and BP1-E 17 neo VI# 4 (L, 10X) ducts.

Sensitivity to anti Fas antibody. The same clones of BP1-E-17neo cells that were unable to form colonies and formed instead ductules in collagen matrix showed the same sensitive to anti Fas antibody than MCF10F cells (Figure 6). All the clones of BP1-E17neo and BP1-E-11neo that did not lost the transformation phenotypes were also resistant to anti Fas antibody behaving like the parental BP1-E cells. (Figures 6 and 7).

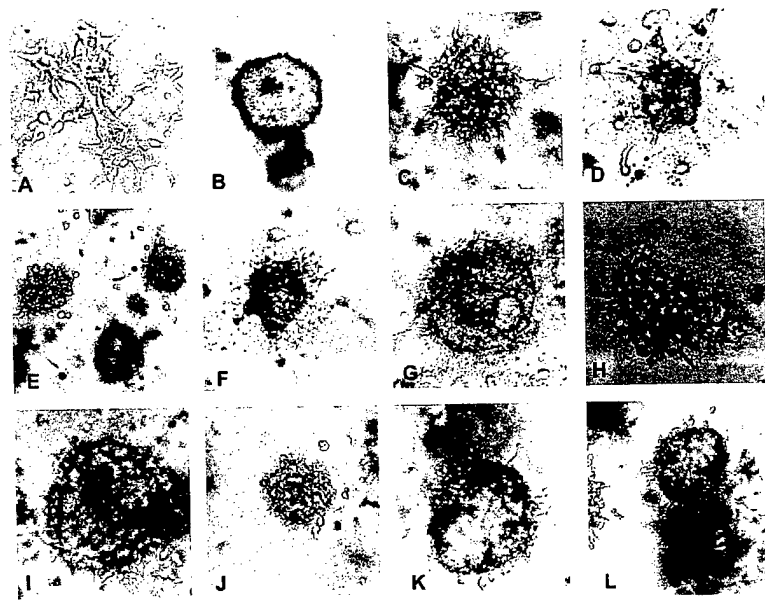


Figure 5: Ductulogenic capacity of chromosome 11 transfected clones in collagen: MCF10F (A, 10X) forms ducts, BP1-E (B, 10X) Ball like structure, BP1-E 11 neo I 1# 1(C, 10X) solid masses, BP1-E 11 neo I 1# 2(D 10X) solid masses, BP1-E 11 neo I 1# 3 (E, 10X) solid masses, BP1-E 11 neo I 1# 4(F, 10X) solid masses, BP1-E 11 neo I 2# 1(G, 10X) solid masses, BP1-E 11 neo I 2# 2(H, 10X) solid masses, BP1-E 11 neo I 3# 1(I, 10X) solid masses, BP1-E 11 neo I 3# 2(J, 10X) solid masses, BP1-E 11 neo I 3# 3(K, 10X) solid masses, BP1-E 11 neo I 3# 4(L, 10X) solid masses.

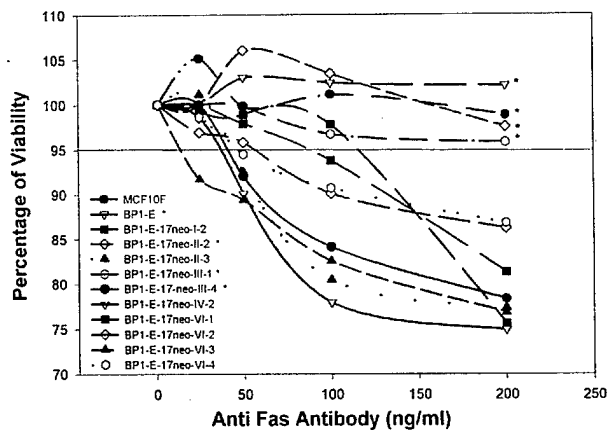


Figure 6: Sensitivity to anti Fas antibody chromosome 17 transfected clones.

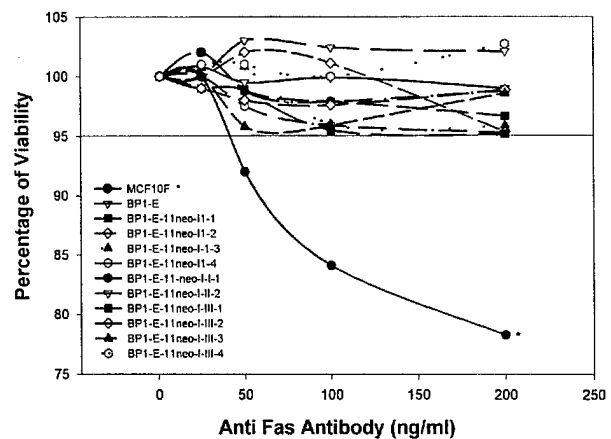
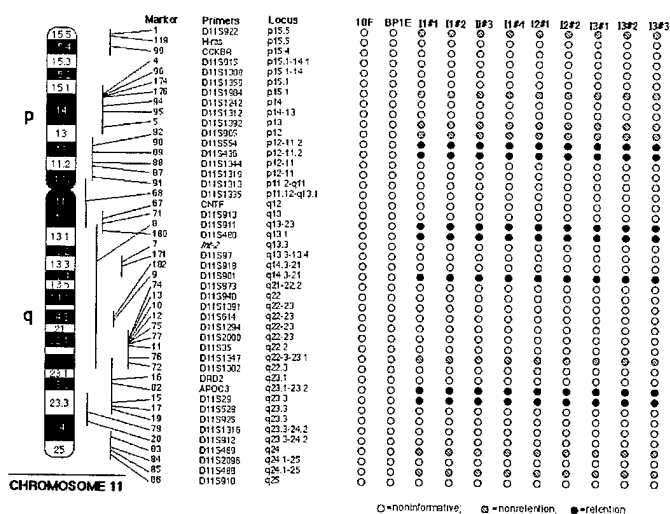


Figure 7: Sensitivity to anti Fas antibody chromosome 11 transfected clones.

Telomerase activity and telomere length. Neither telomerase activity nor the telomere length was affected in the BP1-E cells transferred with Ch 11 or `17.

Confirmation of retention of donor chromosome materials. In order to further determine the specific region(s) of the donor chromosome in the microcell hybrids, 45 micro satellite markers for chromosome 11 (Figure 8) and 41 markers for chromosome 17 (Figure. 9) were analyzed. Among the 45 tested markers for chromosome 11 the following markers showed clear retention D11S554, D11S436, D11S911, D11S480, D11S901, APOC3 and D11S29. Most of the other markers tested were non-informative (Figure 8). From the 41 markers tested on chromosome 17, the following markers showed the retention D17S849, TP53, D17SI852, D17S793, D17S513, D17S796, D17S945, D17SI358, D17S178, D17S791, HGH, D17S722, D17SI150, D17S515, and D17S787. Most of the other markers tested were non-informative (Figure 9). Figure 10 shows the finger print analysis of chromosome 11 transfected clones with markers D11S29 (A), D11S911 (B) and D11S911 (C).

Figures 11A, B, C, and D show the finger print analysis of chromosome 17 transfected clones using the markers, D17S796 (17p13.1-13.2), D17S513 (17p13.1), HGH (17q22-24), D17S849 (17p13.3) and D17S791 (17q21-22). Observe in the panel A the marker D17S796 loci 17p13.1-13.2, of figure 11, that the BP1-E cells has loss a band in the lower allele when compared with MCF10F cells. Transfer of chromosome 17 restore the band in the clones BP1-E17neoI#2, BP1-E17neoII#3, BP1-E17neoVI#2, BP1-E17neoVI#3 and BP1-E17neoVI#4.



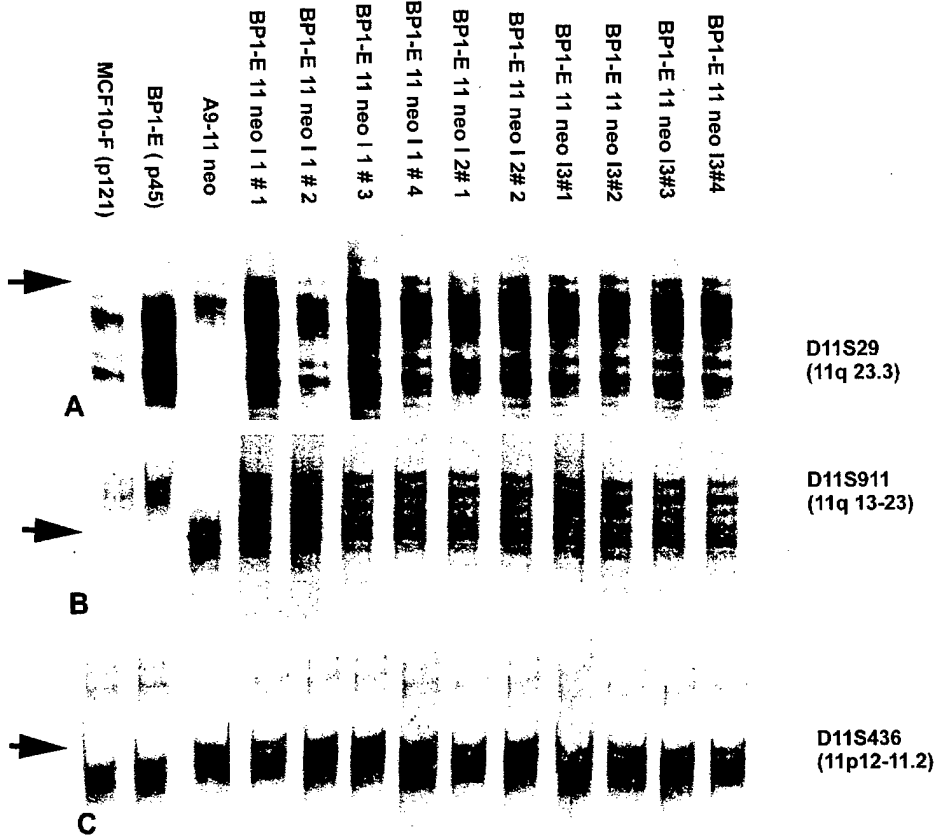


Figure 10: Representative gels showing retention of donor chromosome alleles in BP1E 11 neo clones. Retention of 11q23.3 (D11S29), 11q13-23 (D11S911) and 11p12-11.2 (D11S436) in BP1E 11 neo clones (arrow).

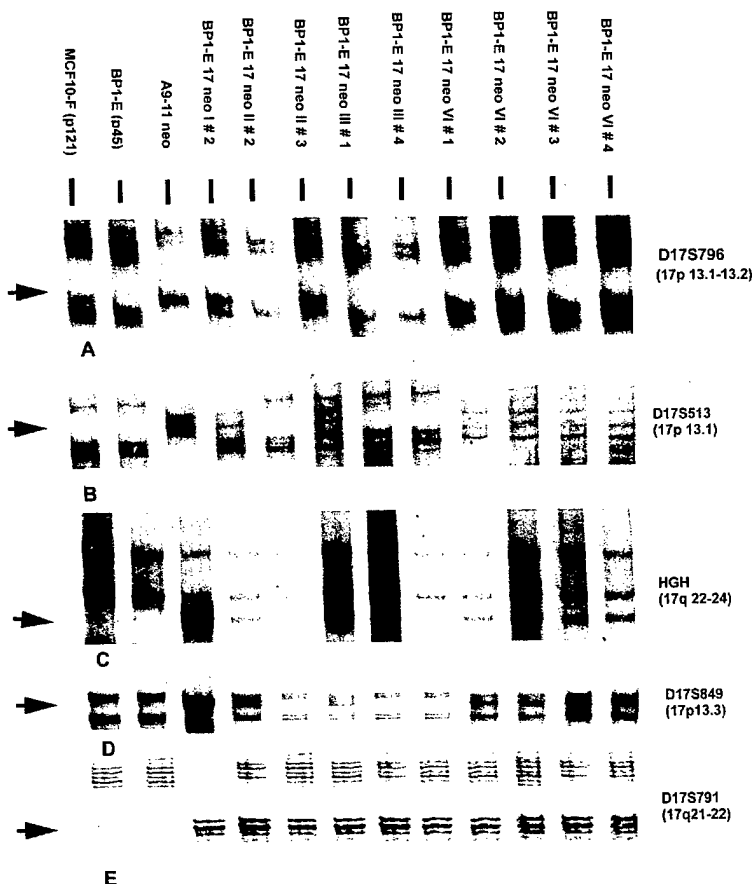


Figure 11: Representative gels showing retention of donor chromosome alleles in BP1E 17 neo clones. Retention of 17p13.1-13.2 (D17S796), 17p13.1 (D17S513), 17q22-24 (HGH), 17p13.3 (D17S849) and 17q21-22 (D17S791) in BP1E 11 neo clones (arrow). Also 12A indicates the loss of genetic material in the process of chemical transformation of MCF10F cells.

Karyotype analysis of BP1-E cells and Fluorescence in situ hybridization (FISH) analysis. Twenty cells were counted, analyzed, and three cells Karyotype. The model chromosome number was 46, and all cell had and abnormal karyotype. The terminal portion of the p arm of chromosome 1 had an unidentified piece of material added. There is a balance translocation between 3p and 9p. Chromosome 11 and chromosome 17 did not show any kind of abnormality in the parental BP1-E cells (Fig. 12).

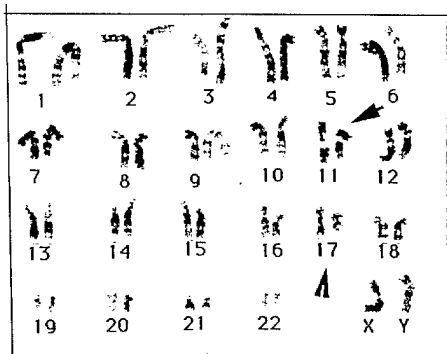


Figure 12: Karyotype analysis of parental BP1E cells to show the normal pair of chromosome 11, 15 and 17.

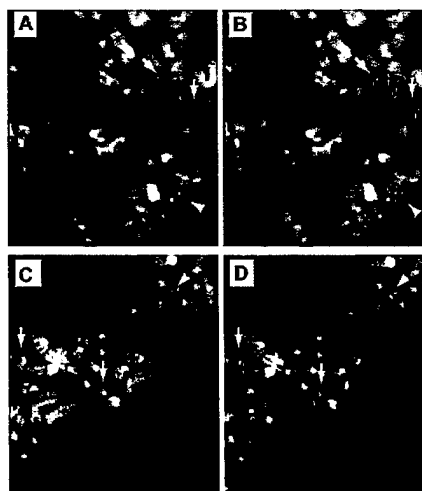


Figure 13: Dual -color fluorescence in situ hybridization (FISH) analysis of BP1E 17 neo II# 3 (Fig 13A & 13B) and BP1E 17 neo III# 4 (Fig. 13C&14D) A and C show the DAPI stained of metaphase with normal pair of chromosome 17(arrow) and additional donor chromosome with pSV2 neo signal (arrowhead), B and D show that in these two clones with chromosome painting probe as well as pSV2 neo signal indicating the transferred chromosome 17(arrowhead) and normal pair of chromosome 17 (arrow).

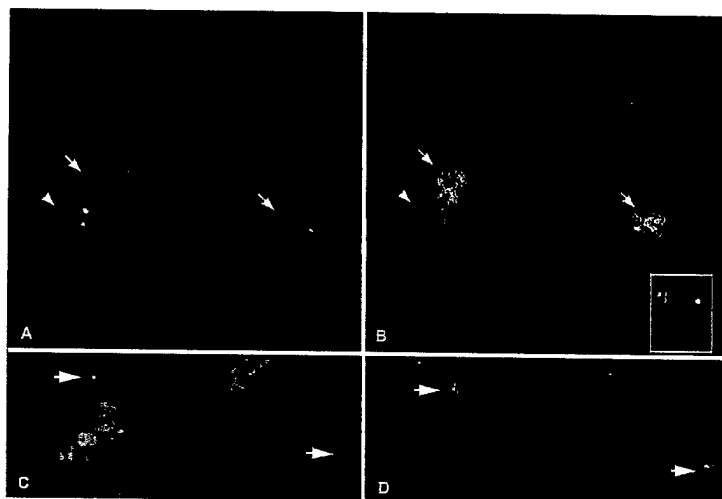


Figure 14: Dual -color fluorescence in situ hybridization (FISH) analysis of BP1E 11 neo I2# 1(Fig. 14A, 14B, 14C & 14D) clone. A and C show the DAPI stained of metaphase with normal pair of chromosome 11(A: arrow), additional donor chromosome 11 with pSV2 neo signal which is translocated to chromosome 15 (A: arrowhead), and additional material of donor chromosome 11 (C: arrow). B and D show that in this clone, with chromosome painting probe as well as pSV2 neo signal indicating the transferred chromosome 11 is translocated to chromosome 15 (B: arrowhead), donor chromosome 11 materials with painting probe (D: arrow) and normal pair of chromosome 11 (B: arrow).

BP1E-17 neo II # 3 clone as a representative for reverted phenotypes clone was randomly picked and FISH analysis performed and we could identify the two normal chromosome 17 and additional

chromosome 17 which was absent in the parental cells with DAPI and painting probe (Figures 13A & 13B). The arrow shows the transferred chromosome with neomycin probe. The transferred chromosome in this clone is rearranged and banding pattern suggests that 17p could be present which would be consistent with the LOH. Fig 13C and 13D show one of the clone (BP1-E 17 neo III # 4) which did not have the reverted phenotype and we could see the normal pair of chromosome 17 and additional small chromosome 17 with neomycin probe (arrow with no head). For chromosome 11 transfected clone we randomly picked one clone (BP1-E 11neo I2#1) and figure 14 shows the DAPI and painting with painting probe for chromosome 11 and neomycin probe. In this clone we could identify the normal pair of chromosome 11 and chromosome 15 consistently showed painting with 11 probe as well as showed signals for the probe for the neomycin marker suggesting that material from the donor chromosome 11 in this clone was translocated to this chromosome 15 (Fig 14A & 14B). Some metaphases did show several fragments of donor chromosome 11 as seen by painting probe (Fig. 14C & 14D).

Discussion: In the present work we show that the immortalized MCF10-F cells are transformed with the chemical carcinogen benz (a) pyrene originating the BP1-E cells. The BP1-E cells express *in vitro* phenotypes indicative of neoplastic transformation such as resistant to Fas mediated apoptosis, advantageous growth, anchorage independence, enhanced chemo invasiveness, and absence of ductulogenic capacity. This neoplastic progression is also associated with MSI at 11q25, and loss of genetic material in 17p13.1 (D17S796)(Figure 11A). Previously our laboratory has successfully transfected 17q25.3 and reverted the transformation and immortalization phenotypes (25). In the work reported here neither transfer of Ch 11 or 17 revert the immortalization phenotype, but Ch 17 induced reversion of the transformation phenotypes including induced Fas mediated apoptosis in 7 out of the 10 clones studied. The reversion of these phenotypes to the one observed in the MCF10 F cells is associated with retention of an allelic portion of chromosome 17 at the p13.1-13.2 locus (D17S796). In the process of transformation by BP the MCF10-F cells loss genetic material in Chromosome 17p13.1-13.2. The seven clones with chromosome 17 transferred that had retained the portion of 17p are the ones with the abrogated transformation phenotypes. This clearly indicates that this locus harbor gene or genes controlling the Fas complex as well as genes that may be associated with the loss of ductulogenic capacity and anchorage indepent growth. Colony formation in agar-methocel assay is a technique utilized as an *in vitro* assay for anchorage independent growth, a parameter indicative of neoplastic transformation. In agar-methocel MCF10-F cells do not produce any colony whereas BP1-E cells produce abundant colony formation with a colony efficiency of 23%. This CE is significantly reduced or abrogated in those clones in which retention of Ch17p13.1-13.2 was found. The clones that did not retain this portion of Ch17 did not change the CE when compared with the parental cells BP1-E. Chromosome 11 transfer is a good control for this study, because it was retained in BP1-E transfected cells but none of the retained portion abrogated the transformation phenotype. The ductulogenic capacity in collagen matrix is other technique by which we could evaluate the cell's ability to differentiate by producing ductular structures. MCF10F cells form ductules in collagen whereas BP1-E cells loss the ductulogenic capacity. This phenotype was also restored in those clones in which Ch17p13.1-13.2 was retained. In a previous publication (25) we have shown that transfer of chromosome 17q25.3(D17S785) was associated with abrogation of the transformation and immortalization phenotypes of BP1-E cells. Immortalization is accepted to be an important event in the carcinogenic process (26,27); the immortalization phenomena involve abrogation of cellular program for limiting the rate and the number of cell replications. Elevated levels of telomerase activity have been detected in a number of immortal cell lines and human tumor tissues (28-33). In addition, abrogation of p53 has been associated with immortalization of HBEC (34,35). In the process of spontaneous immortalization of MCF10F cells the following molecular changes have been detected; balance reciprocal translocation 4 (3; 9)(3p13: 9p22) (16), calcium independence for growing in culture (36) insertional mutation of p53 in exon 7 (35) stabilization of telomere length and over expression of H-ferritin (37). These data led us to determine if the telomerase activity and telomere length were affected

in the BP1-E cells transferred with Ch. 17 or Ch 11 and we did not find significant difference among the cell lines. On the other hand we did observed significant difference in the response of the cells to apoptosis. We have found that MCF10F and MCF10A cells are sensitive to Fas mediated apoptosis whereas (39) BP1E cells are resistant to Fas mediated apoptosis. The relevance of our work in that the abrogation of Fas mediated apoptosis by Ch. 17p13.1-13.2, is that this locus may contain one or more genes controlling the Fas complex. Fas mediated apoptosis plays a major role in apoptosis and the survival of neoplastic cells (38). We have previously demonstrated that in MCF10F cells Fas as well as Fas ligand increased at mRNA as well as at protein level in comparison to BP1-E cells (Unpublished data). The aberration in the signaling pathways leading to apoptosis may result in cancer, autoimmune diseases and inflammatory disorders (40). In view of this, an understanding of the signaling capabilities of apoptosis-inducing death receptors is essential to understand their roles in cell biology and breast cancer initiation as well as in other organ neoplastic process. In addition, mammary involution is associated with degeneration of the alveolar structure as well as programmed cell death of mammary epithelial cells (39). Estrogen exposure represents the major known risk factor for development of breast cancer in women and estrogen and Tamoxifen regulate Fas/FasL expression has been previously reported (38). Chemotherapeutic drugs such as doxorubicin-induces apoptosis through FasL /Fas signaling pathway in proteolytically cleave FasL in tumor cells and induces cell death (41). All these findings by different investigators indicate that Fas mediated apoptosis in breast cancer plays a major role in breast cancer pathogenesis.

In summary our work demonstrates that the chemical carcinogen benz (a) pyrene induces transformation of MCF10F cells as evidenced by anchorage independence; loss of ductulogenic capacity in collagen and loss of activation of Fas mediated apoptosis. These transformation phenotypes are associated with LOH in chromosome 17p13.1-13.2 regions (D17S796). Transfer of chromosome 17 p13.1-13.2 (D17S796) region reverts the transformation phenotypes as well as Fas mediated apoptosis. We postulate that the chromosome 17 (p13.1-13.2) (Locus D17S796) region may contain gene/s responsible for maintaining ductulogenic capacity in collagen, colony formation in agar-methocel and controlling programmed cell death through FAS receptor/ligand complex.

B-iii- *Studies of the function of HEF1 in cell migration.*

Trainee: Sarah J. Fashema, Ph.D.
Mentor: Erica Golemis, Ph.D.
Period reported: July 30,2000 to-October 30, 2000.

Introduction. The multiple stimuli transduced through integrins include external signals provided through contact with the extracellular matrix (ECM) that activate intracellular signaling cascades, and internal changes in the cellular cytoskeleton and signaling apparatus that, conversely, can modulate integrin affinity for extracellular ligands (42). Integrin-dependent signaling contributes to cellular decisions to initiate diverse programs such as proliferation, apoptosis, differentiation, and migration (reviewed in (42-45)). Hence, integrin signaling impacts on a variety of physiological processes including development, tissue remodeling, wound healing, and tumor cell growth and metastasis (46). Efforts to understand the mechanisms through which integrin signaling regulates these cellular processes have focused upon analysis of focal adhesions. Focal adhesion sites consist of integrin receptors clustered following their engagement by extracellular ligand, and an associated complex of intracellular proteins including actin filaments, actin binding and crosslinking proteins, and a number of tyrosine kinases, phosphatases, and docking/adaptor proteins (47). This complex of proteins structurally connects the intracellular actin scaffold to integrins, which serve to tether cells to the basement membrane via binding to ECM ligands. In addition to these physical properties, focal adhesions also act as signaling centers, which generate and convey information from the cell periphery to downstream effector molecules (47). Thus, focal adhesions integrate the mechanical signals derived from morphological changes with the chemical signals triggered by receptor engagement (48-50).

The Cas family of focal-adhesion associated adaptor proteins (reviewed in (51)) includes p130Cas (52), human enhancer of filamentation 1 (HEF1) ((53); also known as CasL (54)), and Efs ((55); also known as Sin (56)). Members of this family were initially identified as components of viral transformation signaling pathways (52,55,57) and/or as modulators of cell growth and morphology (53). Intriguingly, recent clinical studies have indicated that enhanced Cas family expression correlates with significant differences in cancer progression in humans, while induction of p130Cas overexpression enhances resistance to the action of anti-estrogens (58,59). The Cas proteins have a conserved domain structure composed of an amino terminal SH3 domain, a substrate domain which contains multiple tyrosine motifs that are recognized by SH2 domain proteins following phosphorylation, a serine rich region, and a carboxyl terminal dimerization motif (51). HEF1, p130Cas, and Efs localize to focal adhesion sites via interaction of their SH3 domains with FAK (53,60-62), and contribute to the assembly of signaling complexes downstream of the integrin receptor following ligand binding (51). An important question is whether the function of discrete Cas family members at focal complexes is equivalent, or whether individual Cas proteins are associated with promotion of different biological effects.

A number of studies characterizing HEF1 and p130Cas have underscored the potential for functional divergence between these Cas family proteins. HEF1 and p130Cas are differentially regulated; HEF1 is expressed at maximal levels in cells of epithelial and lymphoid origin (53,54,63), whereas p130Cas is expressed ubiquitously (52). Moreover, HEF1 is also regulated in a cell cycle dependent manner and is processed at the G2/M boundary by caspases to truncated isoforms that localize to distinct subcellular compartments (63). Most notably, we have recently found that HEF1 overexpression mediates apoptosis in epithelial derived cell lines including MCF7 and HeLa cells (64), which is contrary to the pro-survival activity described for p130Cas (65,66). Finally, recent studies in lymphoid cells showed that HEF1 (CasL) expression contributes to T cell migration induced by ligation of CD3 and α_1 integrin (67,68).

Taken in sum, these results demonstrate that HEF1 differs from p130Cas both in the manner in which it is regulated and in its spectrum of effector function.

Body. To investigate further these differential properties, we have explored the roles of HEF1 in control of cell shape and motility; moreover, we have begun to elucidate the mechanisms underlying HEF1 induced cellular responses by performing cDNA array analyses to identify downstream transcriptional targets upregulated as a consequence of HEF1 overexpression.

Using a tetracycline-regulated HEF1-expressing MCF7 cell line, we find a dramatic effect of HEF1 overexpression on cell morphology and motility, characterized by development of a crescent shape, enhanced ruffling, and increased cell spreading. HEF1-induced populations contain increased numbers of highly motile cells, and demonstrate increased haptotaxis towards fibronectin. Using DNA array analysis, we find that this enhanced motility is accompanied by upregulation of a set of genes associated with enhanced migration and invasion, including myosin light chain kinase (MLCK), p160ROCK, eight matrix metalloproteinases (MMP)s, and ErbB2. Overall, these data suggest that the spectrum of biological effects attributable to HEF1 is complex, and potentially includes pro-migratory and pro-metastatic activity.

Discussion. The primary goal of the current study was to utilize a well-controlled system to delineate a framework of HEF1-dependent activities that would allow determination of whether HEF1 and Cas acted in parallel, oppositional, or wholly distinct signaling processes in epithelial cells. A second goal was to identify HEF1-responsive targets that might be responsible for mediating the biological functions of the protein.

The initial candidates we have isolated through analyses utilizing cDNA arrays are intriguing. For example, the Rho-associated kinase and effector p160ROCK (69) has been shown to function in the control of cellular motility during developmentally significant processes such as neuronal outgrowth (70). MMPs remodel the ECM by digesting constituent proteins, thereby promoting cellular migration and invasiveness in vivo. These are significant downstream consequences of HEF1 induction, as these proteins are required for many developmental processes and in metastasis of cancerous cells (reviewed in (71,72)). As noted above, MMP1 has previously been reported to be a downstream target of p130Cas activation, induced through the action of CIZ (73). MMP14 (also known as MT1-MMP) is a novel Cas-family target, and of interest because it is a member of a family of structurally distinct, membrane-associated metalloproteases that function both as classical metalloproteases that directly degrade the ECM and as enzymes that cleave and therefore activate other metalloproteases in zymogen form (see (74,75), and discussion therein). Scrutiny of the recently described promoter region of MMP14 (76) reveals at least two matches to the proposed consensus for CIZ binding near the transcriptional start site of the gene, suggesting this transcript may be coordinately regulated with other MMPs. A detected elevation in levels of ErbB2 transcripts is also of considerable interest, insofar as transcriptional upregulation of ErbB2 is a frequent occurrence in, and marker of poor prognosis for, breast cancer (reviewed in (77)). Moreover, ErbB2 overexpression has been shown to promote Cas/Crk coupling and cell invasion (78).

These results emphasize that HEF1 signaling may function in a manner conducive to the promotion of cancer, and may reciprocally regulate the factors shown elsewhere to regulate Cas family members. It is also intriguing to note that HEF1 expression is correlated with an increase in the transcript levels of several genes encoding ECM components (Table I), a finding consistent with that of a recent study that linked the upregulation of a number of ECM proteins to metastatic capacity (79), and also interesting in light of the report that CIZ induces type 1 collagen (80).

Finally, studies this past year provided the first clinical evidence suggesting that perturbation of Cas family expression may result in significant differences in cancer progression in humans (58,59). For example, p130Cas was reisolated as the product encoded by the BCAR1 (breast cancer resistance 1) locus, whose overexpression is associated with resistance to anti-estrogens. Strikingly, the recent cloning of BCAR3, a separate gene with properties similar to BCAR1 in altering response to estrogens, has revealed it to be identical to the molecules AND-34 (81,82) and Nsp2 (83). AND-34 was isolated based

on its interaction with p130Cas and HEF1, while Nsp2 was defined as an adaptor protein linking integrin signaling and JNK activation. Of further interest, the BCAR3/AND-34/Nsp2 proteins are closely related to the CHAT/SHEP1/Nsp3 proteins (83-85) noted above, which are known to interact with the carboxy-terminus of Cas family members and activate JNK signaling. Together with the data in the current study, these findings begin to reveal a profile of Cas-family function that initiates at focal adhesions and ultimately alters the transcriptional regulation of a number of genes, thereby influencing a spectrum of cellular processes related to cancer.

TABLE 1: TRANSCRIPTIONAL RESPONSE TO HEF1

<u>Functional family</u>	<u>HEF1 fold ind.</u>	<u>Vector fold ind.</u>
<u>Motility</u>		
MLCK (U48959)	+, NTH	NS
p160ROCK (U43195)	2.3X	1X
NIK ser/thr kinase (Y10256)	2.7X	1X
PAK alpha (U24152)	+	0.5X
Rho7 (X95456)	+, NTH	0.02X
Rho6 (Y07923)	+, NTH	0.1X
<u>Matrix metalloproteases</u>		
MMP1 (X05231)	+	NS
MMP8 (J05556)	4X	0.8X
MMP9 (J05070; D10051)	1.8X	1X
MMP12 (L23808)	4X	1.4X
MMP13 (X75308)	+	NS
MMP14 (D26512; X83535)	2.5X	1.1X
MMP15 (Z48482)	+	+, NTH
Metalloprotease/disintegrin (U41766)	+	NS
<u>Extracellular Matrix Components</u>		
FN precursor (X02761)	2.7X	NS
collagen 11	2.4X	<0.4X
collagen 4 precursor	+, NTH	NS
cadherin 11 (L34056)	+	<0.5X
cadherin 4 (L34059)	2.2X	0.7X
desmocollin	2.1X	<0.3
heparin sulfate proteoglycan (M85289)	+, NTH	NS
<u>Ephrins and receptors</u>		
ephrin type-A R5 (X95425)	+	0.4X (NTH)
ephrin type-B R1 (L40636)	+, NTH	-, NTH
ephrin type-B R4 (U07695)	+, NTH	-, NTH
ephrin B3	-, NTH	1.1X
eph related ligand (M57730)	+, NTH	0.5X
<u>Oncogenes and related others</u>		
ERB-B2 Receptor	+, NTH	1X (near NS)
MDA7 (U16261)	+	NS
c-fes proto-oncogene (X52192)	+	1.6X

B-iv-Lymphedema Prevention in Breast Cancer Survivors

Trainee: Kerry Sherman, Ph.D.
Mentor: Suzanne Miller, Ph.D.
Period reported: November 1, 2000 – to July 30, 2001

Introduction. The main focus of this research has been developing and designing a study to examine lymphedema prevention in breast cancer survivors. The goal of this proposed research is to explore psychosocial factors associated with the uptake of, and adherence to, precautionary measures to reduce lymphedema risk in breast cancer survivors. Specifically, in this study I will apply the Cognitive-Social Health Information Processing (C-SHIP) model (86,87) to understanding cognitive and affective factors (e.g., knowledge, perceived vulnerability, risk-related affect) that predict uptake of, and adherence to, arm and hand precautions designed to minimize lymphedema risk in breast cancer survivors. In addition, I have participated in writing papers for conferences, and other grant submissions that complement the theoretical framework adopted in the proposed lymphedema prevention study, furthering my understanding of this model, and which allowed me to familiarize with the general medical context at Fox Chase Cancer Center.

Body. My original submission of Statement of Work to the DOD was entitled "An evaluation of the utility of an information intervention for women undergoing diagnostic follow-up of an abnormal mammography test result". This project was conceptualized whilst I was still located in Sydney. However, once I started at Fox Chase Cancer Center, and was able to familiarize myself with the medical system operating within FCCC, I realized that significant procedural, and possibly cultural, differences existed between the Australian and the American breast cancer screening systems. Specifically, this project was designed within a system whereby women attend just one breast cancer screening center, and where this center is responsible for reporting all results of initial mammograms to the women concerned by letter. However, as essentially a secondary referral and treatment facility, Fox Chase Cancer Center does not generally carry out this role. Therefore, my proposed research was not really applicable to the current context, and, in consultation with my mentor, Dr Suzanne Miller, it was decided that this project was not feasible. Consequently, my task was to reformulate my research plans and to come up with a new proposal within the U.S. context at FCCC.

As a result of the changes to my research plan, I contacted Jarsie Weeks at the D.O.D. informing her of my situation. She advised me to resubmit a revised research proposal when such a proposal was reconceptualized. Hence, I have been working on a new research submission that is based on the same theoretical concepts as previously indicated, but now applying these ideas to breast cancer survivors in the context of lymphedema prevention. I have subsequently submitted two grant submissions for funding for the proposed lymphedema research, one submission to the Fox Chase Cancer Center ACS Institutional Grants (February 2001, see Appendix), and one submission to the Susan Komen Foundation. I am currently preparing a grant submission for the lymphedema prevention research to the DOD Breast Cancer Idea Award and co-writing another submission to the DOD Breast Cancer Idea Award for research evaluating the efficacy of an innovative psychosocial counseling intervention for women at hereditary risk for breast and ovarian cancer in relation to decision-making processes regarding participation in genetic testing. The next step to complete in relation to this proposed research is to submit the research protocols for review by the Fox Chase Cancer Center Research Review Committee (RRC) and the Institutional Review Board (IRB). Pending approval from the FCCC review, these research protocols will be submitted to the DOD for IRB approval.

Approximately 20-30% of women develop lymphedema (LE) following breast cancer treatment; this condition has been associated with psychological distress and diminished quality of life. Effective

symptom management requires that women not only recognize early signs of this condition, but that they uptake and maintain precautionary practices over their lifetime. Yet, the limited data available indicate that knowledge and use of symptom minimization precautions are poor, particularly over time. Unfortunately, little is known about how breast cancer survivors perceive their *LE* risk status, and the cognitive-affective factors that promote the uptake of, and adherence to, *LE* symptom minimization precautions. Further, the moderating role of individual differences in attentional style (i.e., *high-monitoring*, which entails attending to, and amplifying health threats, versus *low-monitoring*, which entails distracting from and ignoring such threatening cues) has not been explored. **Objective.** Guided by the Cognitive-Social Health Information Processing (C-SHIP) model, we will conduct a longitudinal study, to assess the barriers and facilitators associated with knowledge about, and initiation and sustained adherence to, *LE* symptom-minimization practices among breast cancer survivors currently unaffected by *LE*. We will explore the mediating role of cognitive-affective variables, and the moderating role of attentional style, on knowledge, uptake and adherence over time. Toward this end, we will survey levels of knowledge, and the practice of symptom minimization precautions at baseline, and again at 6-, and 12-month follow-up. **Specific Aims.** **Aim 1: To delineate the underlying cognitive-affective mediating mechanisms promoting the uptake of *LE* symptom-minimization practices, and their sustained adherence, over time.** Guided by the C-SHIP model, we predict that a woman's pattern of cognitive-affective processing dynamics (i.e., risk perceptions, expectancies, affect and self-regulatory strategies) will impact on her *LE*-knowledge, and uptake of, and adherence to, symptom minimization practices. Specifically, greater *LE*-knowledge, greater intent to establish practices and/or adhere to existing practices, as well as greater uptake of recommendations and sustained adherence will be associated with heightened risk perceptions; greater self-efficacy, greater perceived benefits of, and fewer barriers to, enacting symptom minimization practices; lower *LE*-related distress; and greater ability to perform self-regulatory strategies, over time. **Aim 2: To assess the moderating role of attentional style on the uptake of and adherence to, *LE* symptom minimization practices, over time.** We predict that high monitors will be characterized by heightened perceived *LE*-risk. This focus on risk will also activate increased risk-related distress, lower self-efficacy, and fewer perceived benefits of, and greater barriers to, the uptake of *LE*-precautions. Hence, high monitors will consistently display greater *LE*-related knowledge, and greater intent to adhere to existing practices, and to establish practices, over time, than low monitors, whose level of intent will diminish over time. However, high monitors' focus on threat may undermine their intent to adhere to recommended behaviors; ultimately high monitors may be as non-adherent as low monitors. Thus, we predict that there will be no differences in actual uptake and adherence between high and low monitors, over time. **Study Design.** Women treated for breast cancer, who are presently *LE*-symptom free (N=178), will complete a baseline questionnaire on the day of their post-adjuvant therapy follow-up appointment. Cognitive-affective mediators (e.g., perceived *LE* risk, expectancies and beliefs, risk-related distress, self-regulatory strategies) will be assessed at baseline, and at 6-, and 12-month follow-ups.

In addition to working on the lymphedema research proposal, I have participated in breast cancer related research within the Department of Behavioral Medicine at FCCC. My involvement with this research has furthered my understanding of the medical system in place at FCCC and within the USA, and allowed me to gain a wider understanding of the theoretical underpinnings of the psychosocial breast cancer research currently being conducted at FCCC, thereby assisting my efforts to reformulate my own research directions. Specifically, I have analysed data and written and presented the research findings of a study investigating "Coping style correlates of participation in genetic testing for inherited breast and ovarian cancer risk"(See Appendix) at two conferences: Ethical, Legal, and Social Implications of the Human Genome Project (ELSI) Conference – A Decade of ELSI Research, January 15-17, Bethesda, MD., and the Human Genome Odyssey conference, April 5-8, Akron, OH. I have also co-authored a paper

concerning the role of coping styles in health communications, "Monitoring-Blunting behavioral signatures in coping with health threats: The example of cancer" (See Appendix).

Further, I have analysed data that I collected during 2000 in Australia assessing breast self-examination adherence in young women, and have subsequently written-up these findings for dissemination at the Society of Behavioral Medicine Conference, March 21-24, Seattle, W.A. I have also established connections with Mark Cornfeld, M.D., who is a Consultant on the Pennsylvania Department of Health Work Site Grant. The overall goal of this program is to increase participation in cancer screening and prevention activities. There currently exists an extensive database deriving from four distinct Pennsylvania worksites, from which I plan to obtain, and analyse data pertaining to breast cancer screening adherence (breast self-examination, mammography, clinical breast examination) rates across a spectrum of ages from 20 through to 65. I plan to conduct a comparative analysis of these Worksite data with the Australian data relating to breast screening adherence.

In order to explore the generalizability of the psychological constructs in the breast cancer context, I have co-written the behavioral component of an NIH grant submission for CANCORS relating to monitoring attentional style and decision making processes in metastatic colorectal patients. There is considerable overlap between the constructs investigated in the proposed CANCORS study and that of the research project currently being prepared for submission relating to decision-making processes in women at risk for inherited breast cancer. Specifically, the same overarching theoretical framework, the Cognitive-Social Health Information Processing (C-SHIP) model, has guided the research approach in research proposals relating to breast cancer, and the CANCORS submission. Both the current DOD Breast Cancer Idea Award submission and the CANCORS focus on the relationship between decision-making processes and monitoring attentional style, and C-SHIP mediating variables. While the CANCORS submission focuses on factors that influence treatment decision-making in metastatic colorectal cancer patients, the proposed DOD submission focuses not only on decision making processes, in this case in relation to the decision to undergo genetic testing for *BRCA1/2*, but also the subsequent adherence to screening recommendations, post genetic testing.

B-v.:Polymorphisms of Human UGT1A6 and UGT1A9 Genes and Functional Differences of the Variant Gene Products.

Trainee: Jeffrey Zalatoris, Ph.D.
Mentor: Rebecca Raftogianis, Ph.D.
Period reported: December 1, 2000 – to July 30, 2001

Introduction. Pharmacogenetic studies test the hypothesis that genetic variability of drug metabolizing enzymes is a contributing factor to variation in responses to drugs. UDP-glucuronosyltransferase (UGTs) enzymes are phase II drug metabolizing enzymes that detoxify endogenous and exogenous molecules, allowing for more rapid elimination of the conjugates from the body. Selective estrogen receptor modulators (SERMs) can be biologically inactivated by glucuronidation, and reports suggest that glucuronidation is a method of tamoxifen and raloxifene detoxification (88,89). I have hypothesized that genetic variation in the phenol glucuronidating enzymes UGT1A6 and UGT1A9 may predispose individuals to altered response to antiestrogens or increased risk of estrogen-dependent breast cancer. I have identified four UGT1A6 alleles arising from three common single-nucleotide polymorphisms (SNPs) that encode amino acid changes. I have genotyped healthy, ethnically defined populations for these common genetic polymorphisms of UGT1A6. I have correlated UGT1A6 genotype with the level of UGT biochemical activity in a population of human liver samples. Subsequently, I will biochemically characterize those variants using recombinant wild type and variant UGT1A6 enzymes. Also, I will identify and characterize common genetic polymorphisms in the human UGT1A9 gene in a manner as described for the UGT1A6 analyses. Finally, I will analyze the functional significance of UGT1A6 and UGT1A9 polymorphisms in a cell model system to determine the differential effects of UGT allozymes on the proliferation of the estrogen-dependent cells in the presence of estrogens and antiestrogens. These studies are designed to test the hypothesis that common genetic polymorphisms in UGT1A6 and UGT1A9 result in functional variability at the molecular level. In addition, I hypothesize that the molecular phenotypic variability may have clinical significance, and these genes may be important biological markers for human response to estrogens and antiestrogens.

Body

Identification of UGT1A6 genetic variants. In a population of 35 healthy Caucasians, complete sequencing of the coding region of the UGT1A6 first exon was conducted to identify genetic variants. The first exon was solely examined because this exon, which contains more than half of the coding region for UGT1A6, is unique to UGT1A6, whereas the second through fifth exons of all human UGT1A genes are shared, and therefore are not specific for UGT1A6 enzyme variation. Single nucleotide polymorphisms (SNPs) were detected at UGT1A6 nucleotide positions 19, 315, 541, and 552, and the variants at positions 19, 541 and 552 are non-synonymous, resulting in the following amino acid changes to the enzyme: S7A, T181A, and R184S (Table 1). Within this population, these SNPs were found in four combinations as specific genotypes (Table 1). A PCR-RFLP genotyping assay was developed to rapidly characterize the UGT1A6 genotype in larger populations. Among 65 healthy Caucasians, the four different UGT1A6 alleles were identified in 65%, 27%, 4% and 4% of this population, and the population genotype data conformed to Hardy-Weinberg Equilibrium (Table 2) (90).

Correlation of UGT1A6 genotype and function in liver tissues. To test the initial hypothesis that genotype variation leads to phenotypic differences of UGT1A6, archived human liver tissue samples collected from surgical liver resections performed at the Fox Chase Cancer Center were obtained from the Fox Chase Cancer Center Tumor Bank and studied with IRB approval. From tissue homogenates, DNA was isolated to genotype the UGT1A6 gene, and microsomes containing UGT1A6 protein were isolated

for functional characterization. These samples included 61 tumor tissues and 13 adjacent normal tissues. All tissues were genotyped for UGT1A6 and stratified as "normal" or "tumor" (Table 3). Distribution of alleles within the tumor population also adhered to Hardy-Weinberg Equilibrium; however, the probability function (p) for tumor tissues was 0.11, less robustly supporting Hardy-Weinberg Equilibrium than for the healthy population ($p = 0.86$). The probability of adhering to Hardy-Weinberg Equilibrium is greater as the value of p approaches 1 (90).

Functional assays were developed to study the glucuronidation rate of UGT1A6 toward the highly specific substrate α -naphthol and the modestly specific substrate p -nitrophenol. The assays were developed to detect the spectral absorbance change upon glucuronide conjugation of the substrates. The glucuronidation rates for the two substrates were measured for all isolated microsomes containing UGT1A6. Table 4 and Figure 1 show the UGT1A6 glucuronidation rates as the mean value \pm error for the different genotyped tissues. ANOVA followed by Tukey's test were used to identify significant differences in the mean rate of glucuronidation. A significant difference in glucuronidation rates for both substrates was found for the UGT1A6*2/*2 genotyped microsomes than for the UGT1A6*1/*1, *1/*2, and *1/*3 genotyped microsomes ($p < 0.05$, $p < 0.001$, $p < 0.01$, respectively).

Table 1. Common Nucleotide and Amino Acid Variations of the UGT1A6 First Exon in 65 Healthy Caucasians

Allele	Nucleotide (Amino Acid)								Allele Frequency (n=130 alleles)
	19	(7)	315	(105)	541	(181)	552	(184)	
1	T	(Ser)	A	(Leu)	A	(Thr)	A	(Arg)	0.648 (85)
2	G	(Ala)	G	(Leu)	G	(Ala)	C	(Ser)	0.273 (35)
3	G	(Ala)	A	(Leu)	A	(Thr)	A	(Arg)	0.039 (5)
4	G	(Ala)	G	(Leu)	A	(Thr)	C	(Ser)	0.039 (5)

Allele frequencies were determined from a population of 65 healthy Caucasian subjects by either DNA sequencing or application of the PCR-RFLP assay.

Table 2. UGT1A6 Genotype Frequencies in Healthy Caucasian Population (n= 65) Follow Hardy-Weinberg Equilibrium

Genotype	Observed		Predicted	
	#	Frequency	#	Frequency
*1/*1	28	0.431	28	0.428
*1/*2	24	0.369	23	0.352
*1/*3	2	0.031	3	0.050
*1/*4	3	0.046	3	0.050
*2/*2	4	0.062	5	0.072
*2/*3	2	0.031	1	0.020
*2/*4	1	0.015	1	0.020
*3/*4	1	0.015	0	0.003

Allele frequencies shown in Table 1 were used to calculate predicted values for Hardy-Weinberg Equilibrium. The Hardy-Weinberg Distribution program HWDIAG p1.0 was used to calculate the probability that these data fit the Hardy-Weinberg Equilibrium (90). The threshold for significant variance from Hardy-Weinberg Equilibrium was chosen as 0.05, and as the p value (probability) approaches 1, the probability becomes greater that these data adhere to Hardy-Weinberg Equilibrium. These data had a p value of 0.86, suggesting that the genotype frequencies of this population were distributed in accordance with Hardy-Weinberg Equilibrium.

Recombinant enzyme expression. Recombinant proteins will be examined to further test the hypothesis that the genotypes result in functionally different phenotypes for UGT1A6. The pBlueBac/UGT1A6 vector construct, containing the UGT1A6*1 allele, was kindly provided by Dr. R. Tukey (University of California – San Diego). The gene was digested from the vector and ligated into a histidine-tagged baculoviral expression vector, pBlueBacHis2A. Site-directed mutagenesis was performed on the gene to generate the other three haplotypes of the UGT1A6 gene in the same vector, and these UGT1A6 constructs contained no spurious mutations as detected by complete DNA sequencing. After four unsuccessful attempts to express protein in the insect cell/baculovirus system, the UGT1A6 alleles were recloned into the pTriEX vector to express protein in any of three systems: bacteria, baculovirus/insect cells, and mammalian cells. Experiments to express the gene products in baculovirus/insect cells were again unsuccessful. Expression of UGTs in stably transfected mammalian cells has been successfully accomplished by other investigators (88,91,92). Therefore, I will express the recombinant UGT1A6 proteins by stable transfection of the pTriEX/UGT1A6 variant constructs into mammalian cells that lack endogenous UGTs.

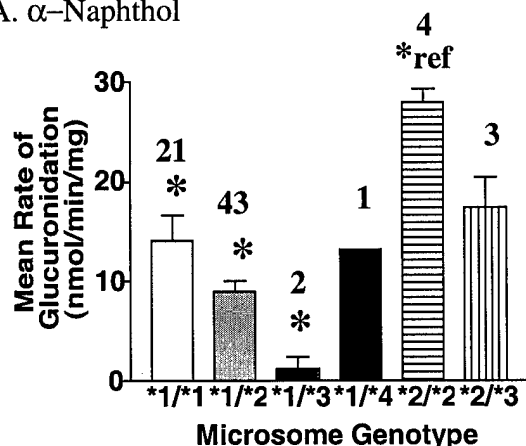
Table 3. UGT1A6 Genotype Frequencies for Liver Tumors

Genotype	Observed (n=62)		Predicted	
	#	Frequency	#	Frequency
*1/*1	19	0.306	23	0.376
*1/*2	35	0.564	26	0.425
*1/*3	2	0.032	2	0.039
*1/*4	1	0.016	1	0.010
*2/*2	3	0.048	7	0.120
*2/*3	2	0.032	1	0.022
*2/*4	0	0.000	0	0.006
*3/*4	0	0.000	0	0.000

Allele frequencies were used to calculate the predicted values for Hardy-Weinberg Equilibrium. Using HWDIAG p1.0 (3), these data had a p value (probability) of 0.11, greater than the threshold for significant variance from Hardy-Weinberg Equilibrium (0.05) thus implying that the population distribution of these genotypes was in equilibrium.

Table 4. Glucuronidation Rates of α -Naphthol and p-Nitrophenol by UGT1A6 Genotype

UGT1A6 Genotype	(#)	Rate of α -Naphthol Glucuronidation	Error (+/-)	Rate of p-Nitrophenol Glucuronidation	Error (+/-)
*1/*1	(21)	13.9	2.63	4.35	0.89
*1/*2	(43)	9.30	1.04	2.54	0.42
*1/*3	(2)	1.18	1.18	0.39	0.31
*1/*4	(1)	13.1	N/A	0	N/A
*2/*2	(4)	28.0	1.25	9.60	1.04
*2/*3	(3)	13.0	4.77	6.42	2.46

A. α -Naphthol

B. p-Nitrophenol

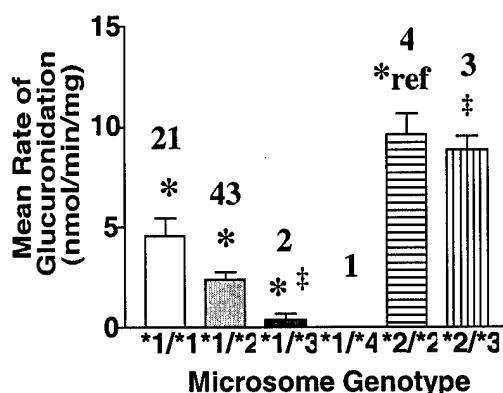


Figure 1. UGT1A6*2 homozygous liver microsomes glucuronidate simple phenols at greater rates than samples that were homozygous for UGT1A6*1 or heterozygous for *1/*2 or *1/*3. Mean glucuronidation rates of α -naphthol (A) and p-nitrophenol (B) were determined by spectrophotometric analyses. The number of liver microsomes associated with each genotype are listed above each error bar. ANOVA followed by Tukey's comparison test was performed. In both A and B, each of the asterisked bars represented significantly different mean glucuronidation rates for these genotyped samples from the mean rate of UGT1A6*2/*2 (*ref): *1/*1, *1/*3, and *1/*2 ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively). In B, the mean glucuronidation rate of p-nitrophenol by UGT1A6*1/*3 was significantly different from that of the UGT1A6*2/*3 ($p < 0.05$)(‡).

Discussion. Four single nucleotide polymorphisms in human UGT1A6 have been shown to exist in four combinations (Table 1). These four alleles are present in 65%, 27%, 4%, and 4% of the healthy Caucasian population analyzed. Archived liver tissues were obtained from persons who had undergone surgical resection for liver cancer. These samples were genotyped and rates of glucuronidation for two substrates of UGT1A6 were measured. ANOVA indicated significant variations in the glucuronidation rates for the tissues with the UGT1A6*2/*2 genotype compared to those tissues with the *1/*1, *1/*2 and *1/*3 genotypes. Notably, a 20-fold difference in glucuronidation rates was observed between liver tissues expressing UGT1A6*1/*3 versus those only expressing UGT1A6*2. The initial hypothesis that genotype variation would result in functional variants was supported by this experiment. These analyses will be verified by studying the glucuronidation rates of recombinantly expressed UGT1A6 allozymes. Study of all of the variant allozymes may provide critical insight about which of the polymorphic amino acids are critical for phenotypic differences. At this time, all attempts to generate the protein in an insect cell/baculovirus system have been unsuccessful. Therefore, stable transfection experiments are underway to express recombinant protein in mammalian cell lines, such as V79 or COS-1, which have been used previously to successfully express other UGTs (88,91,92).

B-vi- Roles of γ -synuclein in breast cancer progression and metastasis

Trainee: Zhong-Zong Pan, Ph.D.
Mentor: Andrew Godwin, Ph.D.
Period reported: January 29, 2001 – to July 30, 2001

Introduction. The synucleins (α , β , γ , synoretin) are a family of small cytoplasmic proteins that are predominantly expressed in neurons. Although α -synuclein is known to play an important role in neurodegenerative diseases, in particular Parkinson's disease, the function of synucleins is unknown. Recently our laboratory and others found that one member of the family, γ -synuclein, is expressed in the majority of late-stage breast (70%) and ovarian carcinomas (> 85%) and some chemoresistant colorectal cancer cell lines, but it is not expressed in normal mammary and ovarian epithelium (93-95). Our laboratory also observed that expression of γ -synuclein induces a phenotype similar to that induced by activation of RhoA/Rac/CDC42, altering the appearance of focal adhesions and stress fibers, and enhancing motility (as observed by time-lapse photography) and invasion (as determined using a Boyden chamber assay). Recent studies have also shown that when γ -synuclein is overexpressed in a breast tumor derived cell line, the cells experience a dramatic augmentation in their capacity to metastasize *in vivo* (96). Taken together, these data strongly suggest the critical role of γ -synuclein in cancer progression and metastasis. Our working hypothesis is that γ -synuclein may be a proto-oncogene, and that the abnormal expression of this protein (i.e., oncogenic form) in breast and ovarian tumors contributes to the metastatic spread and high morbidity associated with advance stages of these diseases. The general goal of this study is to address the molecular mechanisms underlying the function of γ -synuclein in breast and ovarian cancer progression and metastasis. We initially proposed the following specific Aims: 1. *Determine if γ synuclein promotes a motile phenotype in breast tumor cells by modulating the RhoA/Rac/CDC42 signal transduction pathway.* 2. *Identification of proteins interacting with γ -synuclein, and functional characterization of their interaction.*

Body. In the last five months we have made some substantial progress on both aims as described below. Synuclein proteins exhibit a very weak homology to the 14-3-3 family of cytoplasmic chaperone proteins (97,98). The 14-3-3 family of proteins helps regulate many different signal transduction pathways, and is thought to act by directly binding to various protein kinases and bringing them into close proximity with substrate and regulatory proteins. We have recently demonstrated a novel interaction of γ -synuclein with several different mitogen-activated kinases (MAPKs), e.g. extracellular signal-regulated protein kinases (ERK1/2) and c-Jun NH₂-terminal kinase-1 (JNK1), in a single complex (Figure 1).

In addition to its well-known function as a cell survival factor, ERK is also involved in cell migration (9,100). In cells starved for serum or treated with U0126, a MEK1 inhibitor, interaction between γ -synuclein and ERK1/2 is not affected (Figure 2) suggesting that the interaction of γ -synuclein and ERK is independent of the activation status of ERK. Strikingly, our lab recently found that levels of activated Rac (GTP-bound) are consistently increased in tumor cells that over-express γ -synuclein. As activated ERK1/2 specifically localize to focal adhesions, and ERK1/2 has been shown to enhance migration, these results raise the possibility that γ -synuclein may enhance the metastatic potential of tumors through the activation of Rac within the Rho signaling pathway based on protein interactions at focal adhesions. The functional relationships among γ -synuclein, Rac, and ERK will be further studied.

We have shown that exogenous expression of γ -synuclein in tumor cell lines leads to the dramatic down-regulation of SAPK/JNK1 activation in response to a variety of environmental insults (including UV

radiation, heat shock, and sodium arsenate) (Figure 3). The down-regulation of JNK activation by UV is specific to γ -synuclein, since α - and β -synucleins have no effect (Figure 4).

The JNK signaling pathway contributes to multiple biological processes and represents an important mechanism that is used by cells to respond to extracellular stimulation (101). In particular, JNK is activated by a variety of stress signals and *Jnk*^{-/-} cells are defective in stress induced apoptosis. Some chemopreventive drugs also take effect through activating JNK-mediated apoptosis (102). JNK may also be involved in Rho/Rac/Cdc42_MEKK1/4_MKK4/7_JNK signal pathway (103-110), and the JNK activating kinase MKK4 is a metastasis suppressor (110). Based on these evidences we hypothesize that γ -synuclein contributes to carcinogenesis by suppressing stress induced apoptosis and promoting cell migration as a result of altering JNK activation.

Interaction of γ -synuclein with ERK and JNK

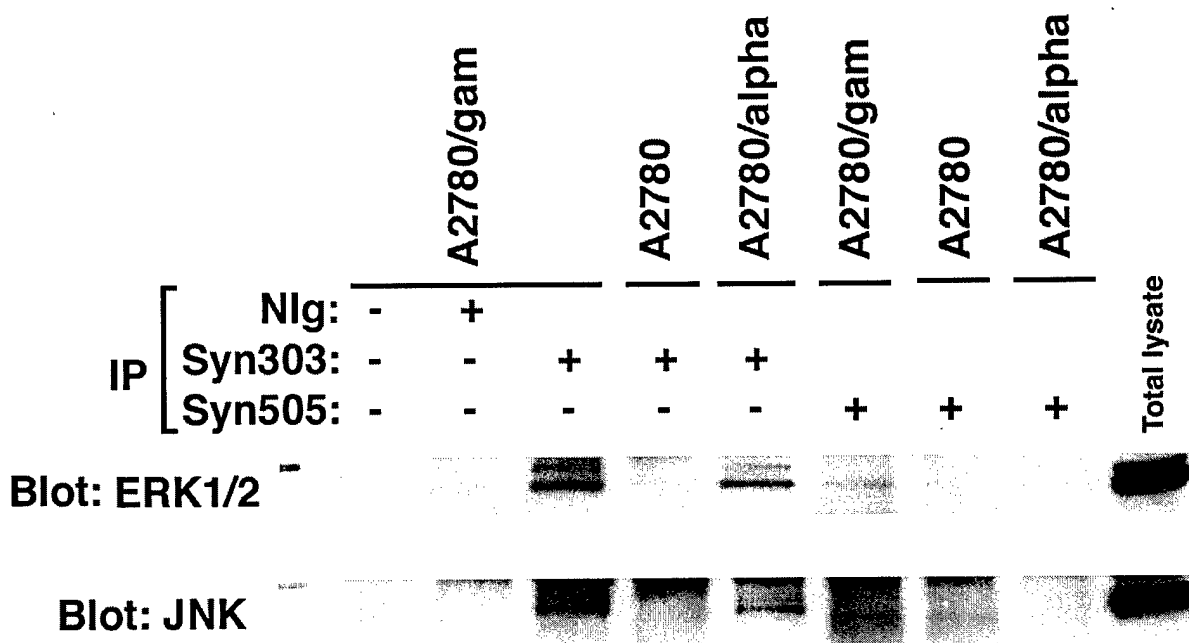


Figure 1. Interaction of γ -synuclein with ERK and JNK. Cell lysates of A2780, A2780/gamma overexpressing γ -synuclein, and A2780/alpha overexpressing α -synuclein were immunoprecipitated with control normal IgG and anti-synuclein antibodies Syn 303 and Syn 505. The proteins in the immuno-complex were checked by immuno-blotting with anti-ERK and anti-JNK antibodies respectively.

When analyzed in A2780 cells for UV induced apoptosis, we found no difference between parental cells vs. those cells with γ -synuclein expression (Figure 5). The reason is unclear yet. Cell survival and cell death depends on the counterbalances between the survival factors and death factors. One possibility could be that the JNK death pathway is overridden by the survival signaling. In this regard, we will test the activation of the survival signaling kinases including ERK-RSK and Akt. We also will dissect what step of the UV-JNK-cytochrome C-caspase 9-caspase 3 pathway is affected by γ -synuclein. UV activated ERK is littlely affected by γ -synuclein (Figure 6), indicating that ERK is not a factor here. Another possibility is that the effect of γ -synuclein depends on the levels of the stress. Indeed, we found that

down-regulation of JNK activation by UV is dosage dependent (Fig. 5). We will test the response of JNK to a variety range of UV dosage, as well as the effect on apoptosis. The other possibility is that the JNK-activated caspase pathway is defective in A2780 cells. From the current data, we do not have a clear answer and we will clarify this question in the next step. In addition, we will test the effects of γ -synuclein on apoptosis in breast cancer cell lines known to undergo apoptosis when treated by UV irradiation.

Interaction of γ -synuclein with ERK is not affected by MEK1 inhibitor (U0126) or serum starvation

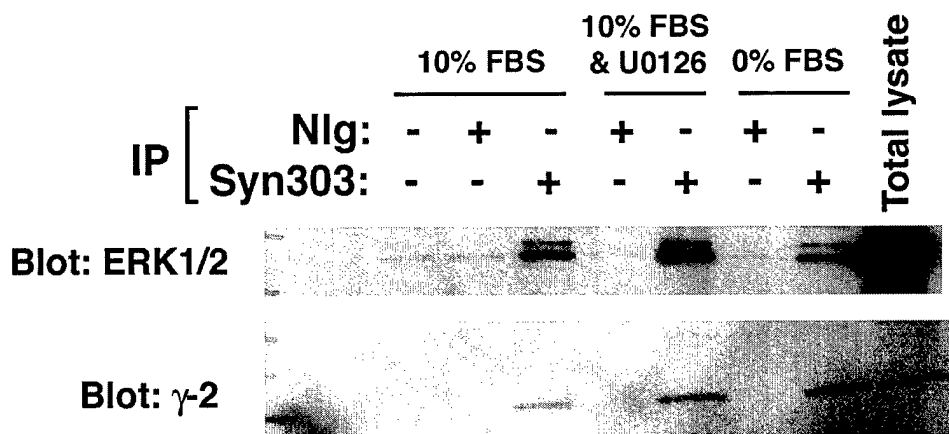


Figure 2. Interaction of γ -synuclein with ERK is not affected by MEK1 inhibitor (U0126) or serum starvation. To block ERK activation, A2780/gamma cells were treated with U0126 (10 μ M) or starved for more than 24hr before lysis and immunoprecipitation. The proteins in the control normal IgG (Nlg) and Syn 303 were checked by immunoblotting with anti-ERK and anti- γ -synuclein (γ -2) antibodies.

Down-regulation of UV-induced JNK kinase activity by γ -synuclein

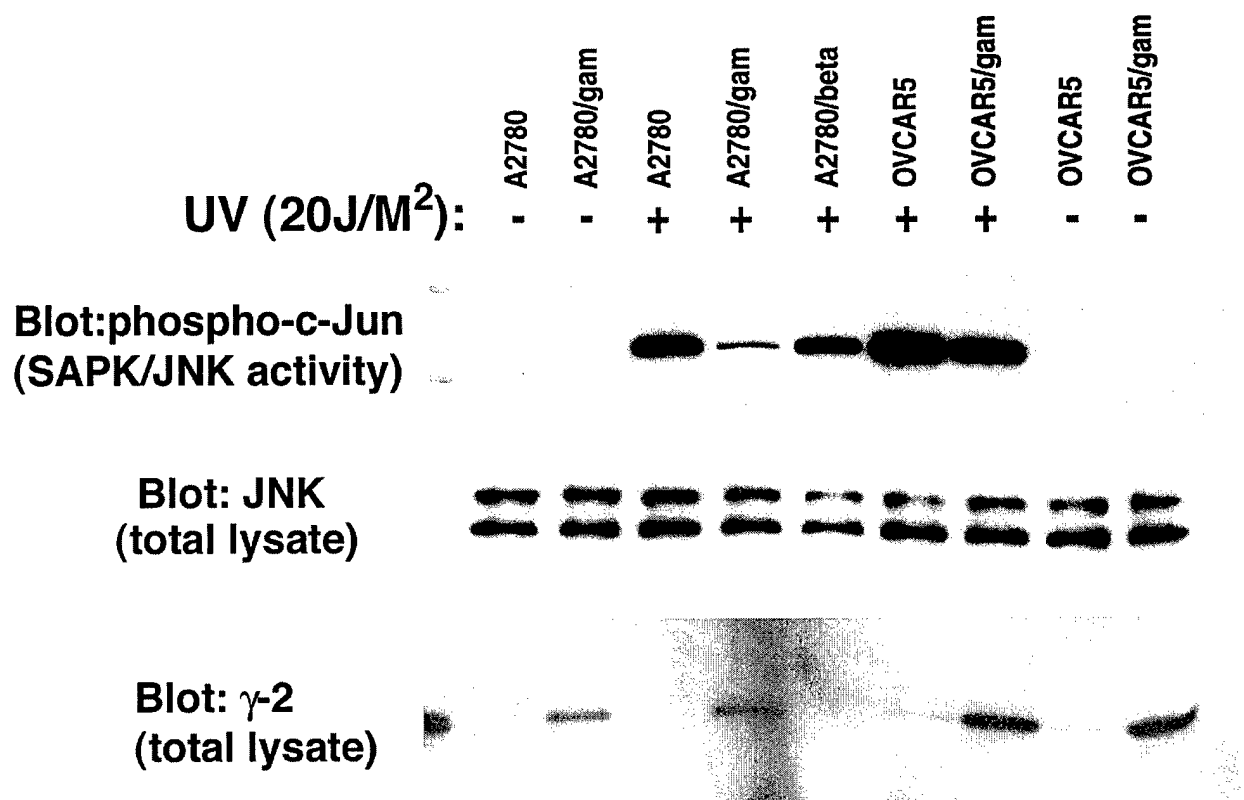


Figure 3. Down regulation of UV-induced JNK activity by γ -synuclein. A2780, A2780/gam, OVCAR5, OVCAR5/gam cells not treated or treated with UV (254 nm, 20J/M²), lysed and analyzed for JNK kinase activity toward c-Jun. The JNK and γ -synuclein protein levels in the lysate were checked by immunoblotting with anti-JNK and γ -2 antibodies.

Down-regulation of UV-induced JNK kinase activity is γ -synuclein specific and UV-dosage dependent

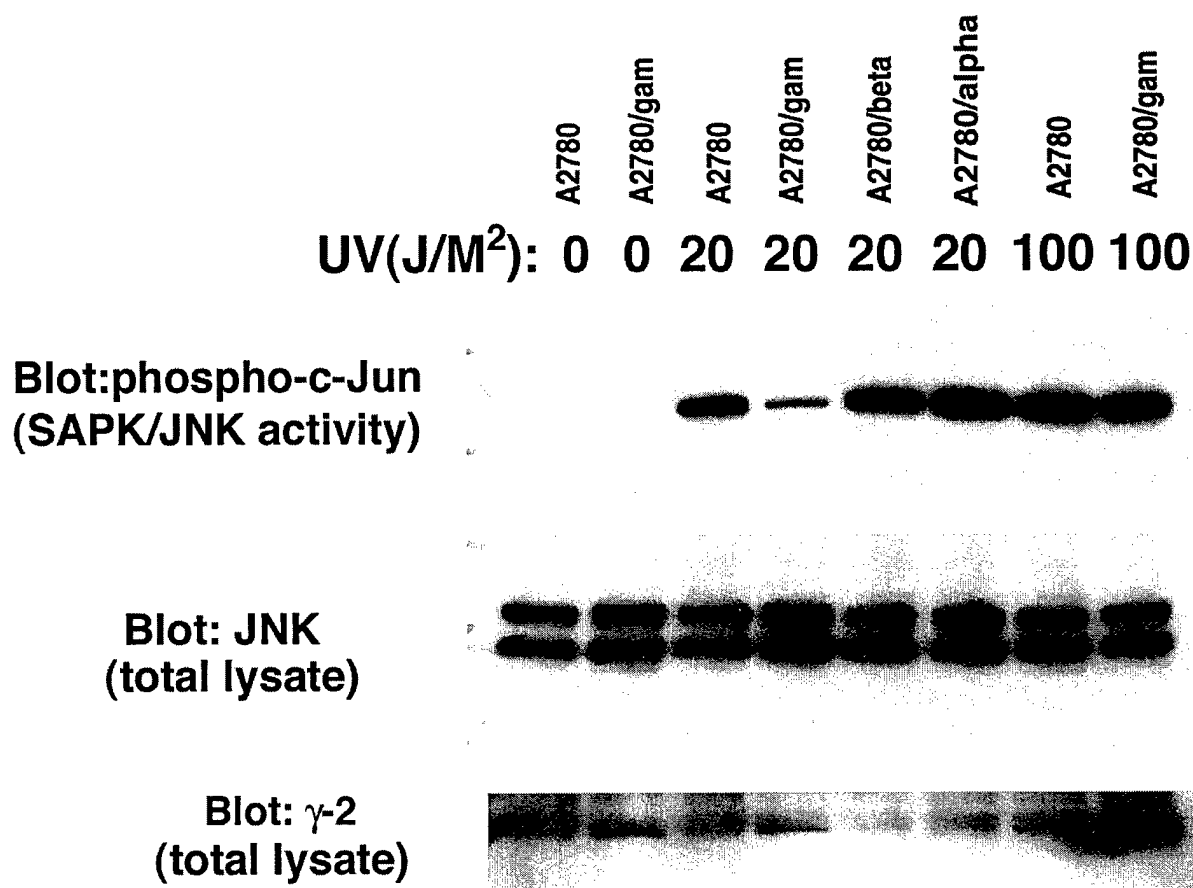
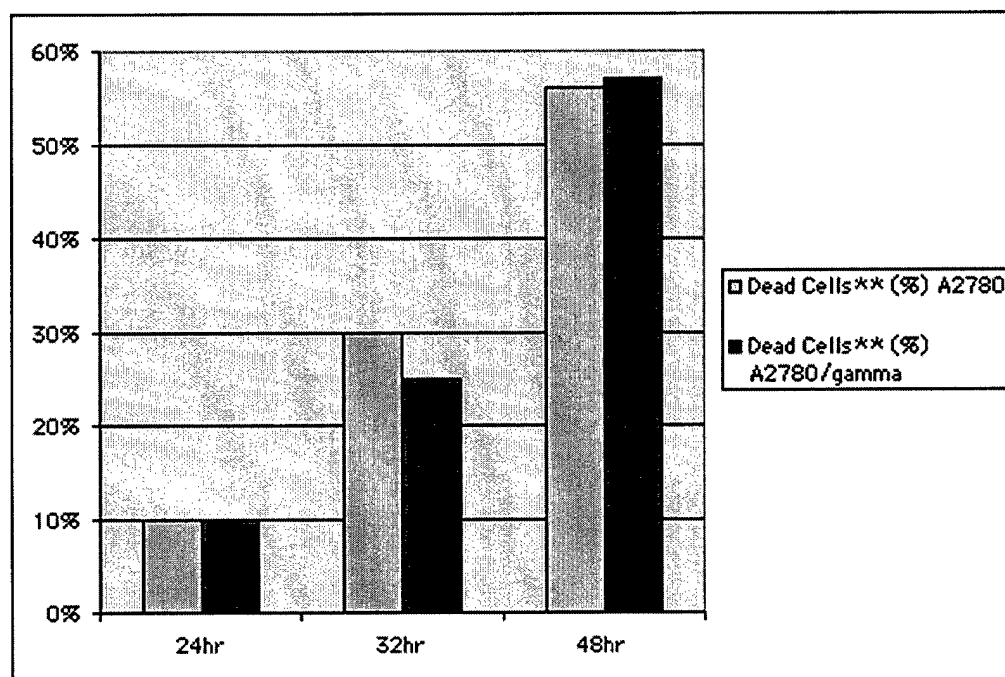


Figure 4. Down regulation of UV-induced JNK kinase activity is γ -synuclein specific and UV-dosage dependent. A2780, A2780/gam, A2780/beta, and A2780/alpha cells, not treated or treated with UV (254 nm, 20J/M² or 100J/M²), were lysed and analyzed for JNK kinase activity toward c-Jun. The JNK and γ -synuclein protein levels in the lysate were checked by immunoblotting with anti-JNK and γ -2 antibodies.

UV induced cell death in A2780 and A2780/gamma cells



Time points*	Dead Cells** (%)	
	A2780	A2780/gamma
24hr	10%	10%
32hr	30%	25%
48hr	56%	57%
* hrs afte UV treatment (J/M2);		
** Trypan blue staining.		

Figure 5. UV-induced cell death in A2780 and A2780/gamma cells. A2780 and A2780/gamma cells were UV-irradiated (20J/M^2), and the cells were harvested at different time points and cell survival was checked with trypan blue staining.

Effects of synucleins on ERK phosphorylation

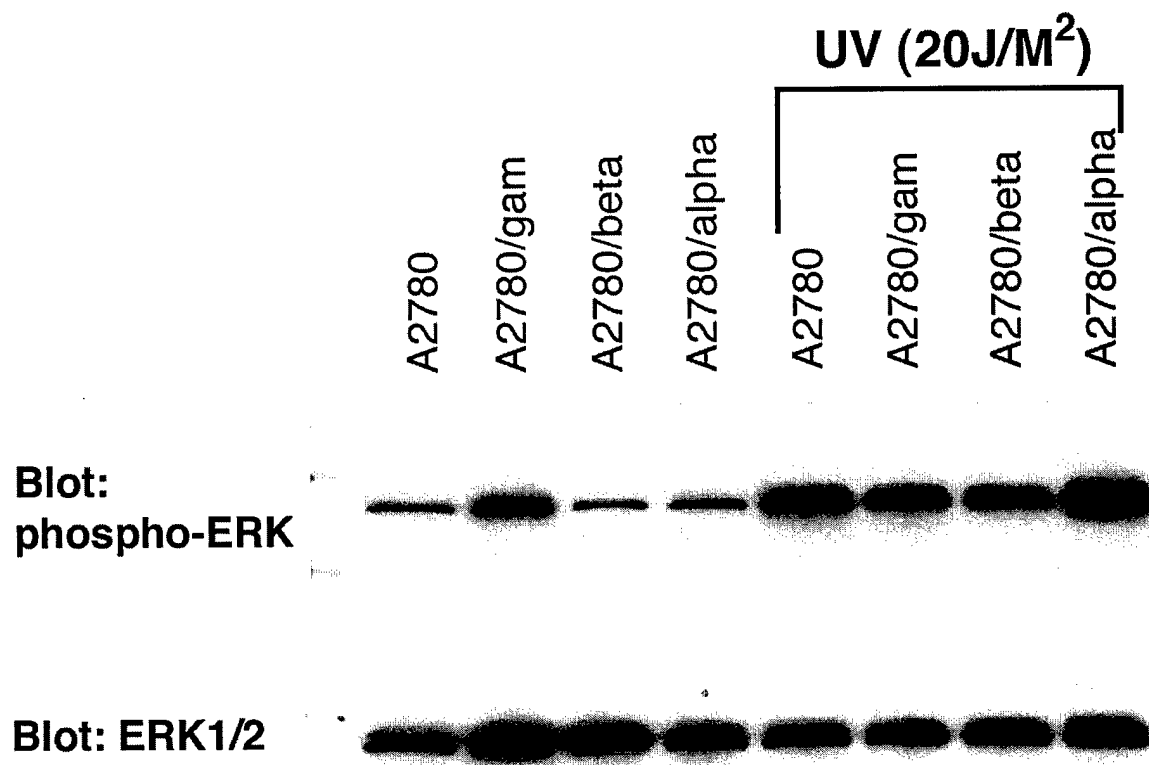


Figure 6. ERK phosphorylation in UV-irradiated cells. A2780, A2780/gam, A2780/beta, and A2780/alpha cells, not treated or treated with UV (254nm, 20J/M²), were lysed and analyzed for ERK phosphorylation by immunoblotting with the antibody specific for phosphorylated ERK.

C- KEY RESEARCH ACCOMPLISHMENTS.

C-I-Study of the functional role of chromosomes 11, and 17 in the process of immortalization and transformation of human breast epithelial cells. By: Hasan, M. Lareef, MD

- a)Transfer of chromosome 17 in to chemically transformed human breast epithelial cells (BP1-E) reverts the transformation phenotypes.
- b)Transfer of chromosome 17 in to chemically transformed human breast epithelial cells induces Fas mediated apoptosis.
- c) Chromosome 11 does not revert the transformation phenotypes in chemically transformed human breast epithelial.
- d)Benz (a) pyrene transformation of MCF10F cells induces the LOH in chromosome 17 p13.1-13.2 (D17S796).
- e)Gene controlling Fas mediated apoptosis may be located in chromosome 17p13.1-13.2(D17S796).

B-ii. Studies of the function of HEF1 in cell migration. By: Sarah J. Fashema, Ph.D.

- a)HEF1 overexpression in MCF7 cell line induces dramatic effect on cell morphology and motility, characterized by development of a crescent shape, enhanced ruffling, and increased cell spreading.
- b)By cDNA array analysis it was found upregulation of a set of genes associated with enhanced migration and invasion, including myosin light chain kinase (MLCK), p160ROCK, eight matrix metalloproteinases (MMP)s, and ErbB2

B-iii. Lymphedema Prevention in Breast Cancer Survivors. By: Kerry Sherman, Ph.D

- a)Written two grant submissions for lymphedema prevention research
- b)Analysed two datasets - Breast Self Examination and BRCA1/2 Testing Informed Consent studies
- c)Written and presented five research papers.
- d)Written a paper for publication, currently in press.

B-iv. Polymorphisms of Human UGT1A6 and UGT1A9 Genes and Functional Differences of the Variant Gene Products. By: Jeffrey Zalatoris, Ph.D.

- a)4 human UGT1A6 genotypes were identified as variant combinations of 4 single nucleotide polymorphisms.
- b)Genotype assay (PCR-RFLP) was developed for rapid screening of UGT1A6 polymorphisms.
- c)Novel a-naphthol and p-nitrophenol glucuronidation assays were developed and applied to analyze rates of glucuronidation in a bank of human liver tissue.

d)Discovery that UGT1A6 glucuronidation was significantly greater for tissues with UGT1A6*2/*2 genotype than tissues genotyped as *1/*1, *1/*2, and *1/*3 ($p < 0.05$, $p < 0.001$, $p < 0.01$).

B-v. Roles of γ -synuclein in breast cancer progression and metastasis. By: Zhong-Zong Pan, Ph.D.

a)Identification of ERK1/2 and JNK as protein kinases that interact with γ -synuclein.

b)Determined that the interaction between ERK1/2 and γ -synuclein is independent of the activation status of ERK1/2.

c)Demonstrated that stress-induced (i.e., UV radiation, heat shock, and sodium arsenite) JNK activation can be specifically blocked by γ -synuclein.

D-REPORTABLE OUTCOMES (See Appendix, Exhibit E):

D-i Study of the functional role of chromosomes 11, and 17 in the process of immortalization and transformation of human breast epithelial cells. By: Hasan, M. Lareef, MD

Abstracts

- 1.Lareef, M.H., Tahin, Q., Russo, I.H., Mor, G., Song, J., Mihaila, D, Slater, C.M., and Russo, J. Transfer of chromosome 17(p13.1) to chemically transformed human breast epithelial cells induces Fas-mediated apoptosis. Proc. Am. Assoc. Cancer Res. 42:1475a, 2001.
- 2 Mello, M.L.S., Lareef, M.H., Hu, Y-F., Yang, X.Q., Vidal, B.C. and Russo, J. RNA relocation at mitosis in benz (a) pyrene transformed human breast epithelial cells after microcell mediated transfer of chromosomes 11 and 17. Proc. Am. Assoc. Cancer Res. 42:4781a, 2001
- 3.Lareef, M.H., Tahin, Q., Russo, I.H., Mihaila, D., Tomaz, J., Tosolini, A., Testa, J., and Russo, J. Chromosome 17(p13.1) transfer reverts transformation phenotypes in human breast epithelial cells. Proc. Am. Assoc. Cancer Res. 42:4803a, 2001.
- 4.Mello, M.L.S., B.de Campos Vidal, Lareef, M.H., J. Russo. Changes in chromatin texture in transformed cells as assessed by molecular biology assays and image analysis. 14th International Congress of Cytology May 27-31 Amsterdam-The Netherlands.

Publications

- 1.Mello, M.L.S., Lareef, M.H., Vidal, B.C. and Russo, J. RNA relocation at mitosis in benz (a) pyrene transformed human breast epithelial cells after microcell mediated transfer of chromosomes 11 and 17. (Submitted for publication, Analytical cellular pathology, 2001).
- 2.Mello, M.L.S., Vidal, B.C, Lareef, M.H. and Russo, J. DNA content, texture and nuclear morphology in benz (a) pyrene transformed human breast epithelial cells after micro cell mediated chromosome transfer of chromosome 11 and 17. (Submitted for publication, Analytical cellular pathology, 2001)

Manuscript in preparation.

- 1.M.H. Lareef, J.Song Q. Tahin, I.H. Russo, G.Mor, D. Mihaila, C.Slater, A. Cuthbert, B. Balsara, J. Testa, D.Broccoli, J.V.Grobelny and J. Russo. Microcell Mediated chromosome 17 transfer to chemically transformed Human Breast Epithelial Cells induces Fas mediated apoptosis and reverts the transformation Phenotypes.

D-ii. Studies of the function of HEF1 in cell migration. By: Sarah J. Fashena, Ph.D.

Manuscripts

- 1.Fashena, S.J., Einarson, M.B., O'Neill, G.M., Patriotis, C., and Golemis, E.A. Dissection of HEF1-dependent changes in epithelial shape, motility, and apoptosis. Journal of Cell Science (*in revision*).

D-iii. Lymphedema Prevention in Breast Cancer Survivors. By: Kerry Sherman, Ph.D

Abstracts

1. Miller, S.M., Driscoll, J.L., Rodoletz, M., Sherman, K.A., Daly, M.B., Diefenbach, M.A., Buzaglo, J.S., Godwin, A.K., & Babb, J.S. (2001, January). Coping style correlates of participation in genetic testing for inherited breast and ovarian cancer risk. Poster presented at A Decade of ELSI Research Conference, January 15-17, Bethesda, MD.
2. Sherman, K.A., Mutton, N., Nour, C., Wainwright, S., & de Torres, A. (2001). Predictors of breast self-examination in young women. Rapid Communication Poster, Society of Behavioral Medicine 22nd Annual Scientific Session, March 21-24, Seattle, WA.
3. Miller, S.M., Rodoletz, M., Buzaglo, J.S., Gray, T., & Sherman, K.A. (2001). Monitoring style in low-income minority women at risk for cervical cancer: Cognitive-Social determinants of adjustment and adherence. Symposium Paper, Society of Behavioral Medicine 22nd Annual Scientific Session, March 21-24, Seattle, WA. Annals of Behavioral Medicine, 23(Suppl.), 117.
4. Miller, S.M., Rodoletz, M., Buzaglo, J.S., Gray, T., & Sherman, K.A. (2001). Monitoring attentional style among low-income minority women at risk for cervical cancer: Cognitive-Social determinants of adjustment and adherence. Poster, Society of Behavioral Medicine 22nd Annual Scientific Session, March 21-24, Seattle, WA. Annals of Behavioral Medicine, 23(Suppl.), 189.
5. Sherman, K.A., Miller, S.M., Rodoletz, M., & Driscoll, J. (2001). Attentional style correlates of participation in genetic testing for inherited breast and ovarian cancer. Paper, Human Genome Odyssey conference, April 5-8, Akron, OH.

Manuscripts

1. Miller, S.M., Sherman, K.A., Buzaglo, J.S., & Rodoletz, M. (in press). Monitoring-Blunting behavioral signatures in coping with health threats: The example of cancer. *Psicologia della Salute*.

Grant submissions/funding applied for:

1. Psychosocial correlates of Uptake of, and Adherence to, Lymphedema Risk Minimization Practices in Breast Cancer Survivors, Fox Chase Cancer Center American Cancer Society Institutional Research Grants, February 2001.
2. Predictors of Uptake of, and Adherence to, Lymphedema Risk Minimization Practices in Breast Cancer Survivors, Susan Komen Foundation.

D-iv. Polymorphisms of Human UGT1A6 and UGT1A9 Genes and Functional Differences of the Variant Gene Products. By: Jeffrey Zalatoris, Ph.D.

Abstracts

1-Presentation at the 2001 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, Orlando, FL, March, 2001.

2-"UGT1A6 Genetic Polymorphisms: Identification and Genotype/ Phenotype Analysis from Human Liver Tissues." J.J. Zalatoris, PhD* and R.B. Raftogianis, PhD, Dept. Pharmacology, Fox Chase Cancer Center, Philadelphia, PA.

Publications

1. J.J. Zalatoris and R.B. Raftogianis. Identification of Genetic Polymorphisms in the Human UGT1A6 Gene and Genotype/Phenotype Correlation in Human Liver Tissue. (in preparation)

D-v. Roles of γ -synuclein in breast cancer progression and metastasis. By: Zhong-Zong Pan, Ph.D.

Abstract.

1. Andrey Frolov, Zhong-Zong Pan, Dominique Broccoli, Lisa Vanderveer, Nelly Auersperg¹, Henry Lynch, Mary Daly, Thomas Hamilton, and Andrew K. Godwin Identification of ovarian cancer-associated genes using a HOSE cell transformation model. Ninth SPORE Investigators's Workshop, Washington, DC, July 15-17, 2001".

E-CONCLUSIONS:

E-i.- Study of the functional role of chromosomes 11, and 17 in the process of immortalization and transformation of human breast epithelial cells. By: Hasan, M. Lareef, MD

Our experiments have allowed us to identify the possible locus of gene/genes controlling Fas mediated apoptosis and transformation phenotypes in benz (a) pyrene transformed human breast epithelial cells. We postulate this gene/s is/are located in chromosome 17 p13.1-13.2 regions. Transfer of chromosome 17p13.1-13.2 to BP1E cells reverts the transformation phenotypes.

E-ii.-Studies of the function of HEF1 in cell migration. By: Sarah J. Fashema, Ph.D.

Our findings begin to reveal a profile of Cas-family function that initiates at focal adhesions and ultimately alters the transcriptional regulation of a number of genes, thereby influencing a spectrum of cellular processes related to cancer.

E.-iii. Lymphedema Prevention in Breast Cancer Survivors. By: Kerry Sherman, Ph.D

To date, although a significant subset of women experience lymphedema symptoms following breast cancer treatment, few studies explore the facilitators of, and barriers to, the uptake of symptom minimization practices and their sustained adherence, over time. The information derived from this needs-analysis will allow us to generate theory-guided predictive models of individuals who are most, and least, likely to adopt and adhere to risk-minimization recommendations. These data, in turn, will be used to develop evidence-based psychosocial interventions tailored to the woman's cognitive-affective signature, with a view to facilitating the uptake of, and adherence to, symptom-minimization practices among women who might benefit from these practices.

E.-iv. Polymorphisms of Human UGT1A6 and UGT1A9 Genes and Functional Differences of the Variant Gene Products. By: Jeffrey Zalatoris, Ph.D.

A principal focus of pharmacogenetics is the identification of genetic variants of drug metabolizing enzymes that result in different drug responses in humans. UGT glucuronidation of selective estrogen receptor modulators has led to the hypothesis that phenol glucuronidating enzymes are at least partially responsible for the bioinactivation of SERMs. Because SERMs are being prescribed as long-term preventative therapies against the development of breast cancer, the response of patients to these drugs will likely dictate the patients' adherence to the therapy regimen and the acceptance of any costs in terms of financial and health issues. Therefore the identification of genetic factors influencing responses to these therapies may aid physicians in their ability to prescribe the drugs effectively. This study is aimed at defining whether these putative SERM inactivating enzymes have genetic variations that result in predictable phenotypes at both the molecular level and in the patient.

UGT1A6 genotype has been determined in a Caucasian population and in a population of liver tumor tissues from patients with liver cancers. The study of liver tissue genotype and phenotype was performed because these tissues are rich in UGT1A6 and supplied enough material to compare glucuronidation rates among different genotyped samples. In the study of liver tissue glucuronidation of the highly specific UGT1A6 substrate a-naphthol and the more general UGT substrate p-nitrophenol, the liver microsomes homozygous for UGT1A6*2/*2 showed statistically greater rates of glucuronidation than the microsomes genotyped as *1/*1, *1/*2, and *1/*3. In fact, a 20-fold difference was detected between tissues expressing the UGT1A6*1/*3 allozymes and those that were homozygous for the UGT1A6*2. These

data suggest that the *2 allozyme has a greater specific activity in liver than the *1 allozyme. Because of the rarity of the *3 and *4 genotypes, no homozygous liver tissues have been identified from this population. Therefore, to study the effect of specific activity variation due to the genotype, expression of recombinant proteins for all of these variants will need to be performed. To address the hypothesis that variant allozymes result in functional changes to the inactivation of estrogens and antiestrogens *in vivo*, a cell model study will be conducted to assess the proliferation of estrogen-dependent mammalian cells stably transfected with the variant UGT1A6 genes in the presence and absence of SERMs. Differences in the stability of the UGT1A6 allozymes will also be studied using these cell lines. Finally, the identification of UGT1A9 genetic polymorphisms and the assessment of phenotypic differences of the expressed proteins will lead to a greater understanding of the contributions of UGT1A9 to the metabolic inactivation of SERMs.

E.-v.-. Roles of γ -synuclein in breast cancer progression and metastasis By: Zhong-Zong Pan, Ph.D.

In the present study, we found ERK1/2 are interacting with γ -synuclein independent of the activation status of ERK. Since ERK and CAS Rho are involved in cell migration, and γ -synuclein overexpression enhances cell migration, we will further study the functional relationship among γ -synuclein, CAS Rho, and ERK in cell migration. We also found that JNK is interacting with γ -synuclein. The activation of JNK by a variety of stress signals including UV is down-regulated specifically by γ -synuclein but not by α - and β -synucleins. Since JNK is essential for UV induced apoptosis in a variety kinds of cells, the biological implications of γ -synuclein down-regulation of UV-activated JNK will be studied.

F. REFERENCES

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TITLE: FOX CHASE CANCER CENTER INSTITUTIONAL BREAST CANCER TRAINING PROGRAM
(FCCC-IBCTP)

PRINCIPAL INVESTIGATOR: JOSE RUSSO, M.D., F.C.A.P.

APPENDIX

Exhibit A: Poster Advertisement Breast cancer Training Program

Exhibit B: Advertisement in Science

Exhibit C: Raftogianis Biosketch

Exhibit D: Roster of Lectures for the DOD Breast Cancer Training program

Exhibit E: Publications enclosed:

BREAST CANCER TRAINING PROGRAM

At the Fox Chase Cancer Center

**FOUR POSITIONS ARE IMMEDIATELY AVAILABLE FOR
A TERM OF TWO YEARS**

THE PROGRAM CONSISTS OF 16 EDUCATIONAL MODULES ENCOMPASSING:

- ★ Cellular and Molecular Biology of Breast Cancer
- ★ Cytogenetics
- ★ DNA Repair, Mutation Detection in Breast Cancer Genes, and Modern Technologies of Protein Analysis
- ★ Mechanism of Inherited Breast Cancer
- ★ Genetic and Molecular Basis of Cancer Metastasis
- ★ Regulation of Mitogenic and Apoptotic Signals
- ★ Cyclins and Regulation of Stress Response
- ★ Genetic Complementation
- ★ Drug Resistance and Targeted Immunotherapy
- ★ Genetic Epidemiology and Control of Breast Cancer
- ★ Breast Cancer Prevention
- ★ Psychosocial and Behavioral Medicine in Breast Cancer
- ★ Breast Cancer Diagnosis and Treatment
- ★ Biostatistics
- ★ Library Instruction
- ★ Bioinformatics

TRAINING IN THESE DISCIPLINES WILL BE CARRIED OUT IN THREE PHASES

- PHASE 1** will provide postdoctoral fellows an overall view of the various fields and possibilities available in the program.
- PHASE 2** will provide training in specific Modules for developing a specific research project under a co-mentoring system.
- PHASE 3** will be devoted to data analysis, publications, and evaluation of training efficiency by the Faculty and Advisory Committee.

APPLICATION FOR POSTDOCTORAL FELLOWSHIP

Applications for the training program must include:

1. an application letter addressed to the Program's Director (J. Russo, MD; Director Breast Cancer Training Program; Fox Chase Cancer Center; 7701 Burholme Avenue; Philadelphia, PA 19111)
2. a statement of the fellow's background, training, and professional interests and goals
3. a complete curriculum vitae
4. a minimum of three letters of recommendation

PREREQUISITES

Fellows applying to the program should have a Ph.D. and/or M.D. degree with a background in biology, molecular biology, chemistry, including organic and physical chemistry, mathematics, biochemistry, and genetics.

Fox Chase Cancer Center is committed to a policy of equal opportunity and affirmative action. Decisions concerning admission to the program will be established on the qualifications of the applicant. The Center does not discriminate on the basis of race, creed, color, religion, national origin, sex, age, disability, marital status, sexual orientation, or status as a disabled veteran or veteran of the Vietnam era.

Please send me more information regarding the
BREAST CANCER TRAINING PROGRAM
at Fox Chase Cancer Center.

Name: _____
Address: _____
City: _____
State: _____ Zip: _____
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TITLE: FOX CHASE CANCER CENTER INSTITUTIONAL BREAST CANCER TRAINING PROGRAM
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PRINCIPAL INVESTIGATOR: JOSE RUSSO, M.D., F.C.A.P.

Appendix B.

BREAST CANCER TRAINING PROGRAM
at the Fox Chase Cancer Center

THE PROGRAM CONSISTS OF 16 EDUCATIONAL MODULES
ENCOMPASSING:

- Cellular and Molecular Biology of Breast Cancer
- Molecular Cytogenetics
- DNA Repair, Mutation Detection in Breast Cancer Genes, and Modern Technologies of Protein Analysis
- Mechanism of Inherited Breast Cancer
- Genetic & Molecular Basis of Cancer Metastasis
- Regulation of Mitogenic and Apoptotic Signals
- Cyclins and Regulation of Stress Response
- Genetic Complementation
- Drug Resistance and Targeted Immunotherapy
- Genetic Epidemiology/Control of Breast Cancer
- Bioinformatics Methods
- Breast Cancer Prevention
- Psychosocial and Behavioral Medicine in Breast Cancer
- Breast Cancer Diagnosis and Treatment
- Biostatistics
- Library Instruction

Faculty

K.M. Albert	N. Galpern	S.M. Miller	R. Strich
M.B. Daly	L. Goldstein	B. Patriotis	J.D. Tisdall
J. Dorgan	E.A. Golemls	A. Rogatko	J. Testa
B. Eisenberg	B.A. Lewis	J. Russo	L. Weiner
A. Godwin	F.J. Manion	I.H. Russo	A.T. Yeung

Applications for the training program must include:

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4. A minimum of three letters of recommendation.

Fox Chase Cancer Center is committed to a policy of equal opportunity and affirmative action and decisions concerning admission to the program will be established on the qualifications of the applicant.

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel the order listed on Form Page 2.
Photocopy this page for each person.

NAME Rebecca Blanchard Raftogianis	POSITION TITLE Associate Member
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EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Albany College of Pharmacy, Albany, NY	B.Sc.	1990	Pharmacy
University of Utah, Salt Lake City, Utah	Ph.D.	1995	Pharmaceut. Chem.
Mayo Clinic, Rochester, MN	Postdoc	1995-1998	Pharmacogenetics

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE:

Research and Teaching Assistant, Dept. of Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT	Jun 1990-Jun 1995
Research Fellow, Dept. of Pharmacology, Mayo Clinic, Rochester, MN	Jul 1995-Jun 1997
Instructor, Dept. of Pharmacology, Mayo Clinic, Rochester, MN	Jul 1997-Sept 1998
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Research Associate, Dept. of Pharmacology, Mayo Clinic, Rochester, MN	Jul 1998-Sept 1998
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PROFESSIONAL ASSOCIATIONS

American Association for the Advancement of Science
American Association of Pharmaceutical Scientists
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HONORS AND AWARDS

Rho Chi National Graduate Scholarship	1990
American Foundation for Pharmaceutical Education Predoctoral Fellowship	1991-1993
American Foundation for Pharmaceutical Education – Marion Merrell Dow Industry Oriented Fellowship in Clinical Pharmacy Science	1993-1994
Walter F. Enz-Upjohn National Award for Excellence in Pharmaceutical Science	1994
Postdoctoral Award for Presentation in the Category of <i>In Vivo</i> Applications, International Society for the Study of Xenobiotics	1995
Trainee Investigator Award for Excellence in Scientific Research, Biomedicine '96	1996
Presidential Trainee Award, American Society for Clinical Pharmacology and Therapeutics	1997
NIH Individual Postdoctoral Fellowship	1997-1998

PUBLICATIONS:**Manuscripts**

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DOD Breast Cancer Training Program Seminar Series and Dates of Activities for 2001

<u>Day and Time</u>	<u>Speaker</u>	<u>Theme</u>	<u>Place</u>
Thursday March 29 4:00pm	Dr. Michael H. Torosian	The Role of the Surgeons in the Patient with Breast Cancer	Room A-B
Thursday April 26 4:00 pm	Dr. Irma H. Russo	The Use of Experimental Models for the Study of Breast Cancer	Room A-B
Thursday May 24 4:00 pm	Dr. Joseph Testa	The Genome of Breast Cancer	Room A-B
Friday, June 1	Submitted by H Lareef, J. Zalatoris, K. Sherman, Z. Pan and Dr. E. Golemis	Deadline for Presentation of Progress Report of the Trainees work up to date, sent by e-mail attachment to J Russo.	
Thursday June 28 4:00 pm	Dr. Jose Russo	The Role of the Pathologist in the Diagnosis of Breast Cancer	Room A-B
Thursday July 25 4:00 pm	Dr. Yoshihiro Matsumoto	DNA Repair and Cancer	Room A-B
Thursday August 30 4:00 pm	Dr. Andrew Godwin	Familial Breast Cancer	Room A-B
Wednesday Sept 12 3-5 pm	Trainees	Presentation by Trainees to the Faculty and Advisory Board of work in progress.	Room A-B
Thursday Sept. 27 4:00 pm	Dr. Rebecca Raftogianis	Metabolism of Estrogen and Breast Cancer	Room A-B
Thursday October 25 4:00 pm	Dr. Christos Patriotis	From cDNA Array to Proteomic	Room A-B
Thursday Nov. 15 4:00 pm	Dr. Erica Golemis	Addressing the Role of Complex Proteomic Networks in Cancer Related Signaling	Room A-B
Thursday Dec. 13 4:00 pm	Dr. Randy Strich	The Role of the Stress Response and Breast Cancer	Lippencott Room

TITLE: FOX CHASE CANCER CENTER INSTITUTIONAL BREAST CANCER TRAINING PROGRAM
(FCCC-IBCTP)

PRINCIPAL INVESTIGATOR: JOSE RUSSO, M.D., F.C.A.P.

Appendix E.

Publications enclosed:

1. Lareef, M.H., Tahin, Q., Russo, I.H., Mor, G., Song, J., Mihaila, D., Slater, C.M., and Russo, J. Transfer of chromosome 17(p13.1) to chemically transformed human breast epithelial cells induces Fas-mediated apoptosis. *Proc. Am. Assoc. Cancer Res.* 42:1475a, 2001.
2. Mello, M.L.S., Lareef, M.H., Hu, Y-F., Yang, X.Q., Vidal, B.C. and Russo, J. RNA relocation at mitosis in benz (a) pyrene transformed human breast epithelial cells after microcell mediated transfer of chromosomes 11 and 17. *Proc. Am. Assoc. Cancer Res.* 42:4781a, 2001
3. Lareef, M.H., Tahin, Q., Russo, I.H., Mihaila, D., Tomaz, J., Tosolini, A., Testa, J., and Russo, J. Chromosome 17(p13.1) transfer reverts transformation phenotypes in human breast epithelial cells. *Proc. Am. Assoc. Cancer Res.* 42:4803a, 2001.
4. Mello, M.L.S., B.de Campos Vidal, Lareef, M.H., J. Russo. Changes in chromatin texture in transformed cells as assessed by molecular biology assays and image analysis. 14th International Congress of Cytology May 27-31 Amsterdam-The Netherlands.
5. Mello, M.L.S., Lareef, M.H., Vidal, B.C. and Russo, J. RNA relocation at mitosis in benz (a) pyrene transformed human breast epithelial cells after microcell mediated transfer of chromosomes 11 and 17. (Submitted for publication, Analytical cellular pathology, 2001).
6. Miller, S.M., Driscoll, J.L., Rodoletz, M., Sherman, K.A., Daly, M.B., Diefenbach, M.A., Buzaglo, J.S., Godwin, A.K., & Babb, J.S. (2001, January). Coping style correlates of participation in genetic testing for inherited breast and ovarian cancer risk. Poster presented at A Decade of ELSI Research Conference, January 15-17, Bethesda, MD.
7. Sherman, K.A., Mutton, N., Nour, C., Wainwright, S., & de Torres, A. (2001). Predictors of breast self-examination in young women. Rapid Communication Poster, Society of Behavioral Medicine 22nd Annual Scientific Session, March 21-24, Seattle, WA.
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9. Miller, S.M., Sherman, K.A., Buzaglo, J.S., & Rodoletz, M. (in press). Monitoring-Blunting behavioral signatures in coping with health threats: The example of cancer. *Psicologia della Salute*.
10. UGT1A6 Genetic Polymorphisms: Identification and Genotype/ Phenotype Analysis from Human Liver Tissues." J.J. Zalatoris, PhD* and R.B. Raftogianis, PhD, Dept. Pharmacology, Fox Chase Cancer Center, Philadelphia, PA. Presentation at the 2001 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, Orlando, FL, March, 2001.
11. Andrey Frolov, Zhong-Zong Pan, Dominique Broccoli, Lisa Vanderveer, Nelly Auersperg¹, Henry Lynch, Mary Daly, Thomas Hamilton, and Andrew K. Godwin Identification of ovarian cancer-associated genes using a HOSE cell transformation model. Ninth SPORE Investigators's Workshop, Washington, DC, July 15-17, 2001".

Reprinted from Proceedings of American Association for Cancer Research
92nd Annual Meeting March 24-26, 2001 New Orleans, LA.

#1475 Transfer of Chromosome 17 (p13.1) to Chemically Transformed Human Breast Epithelial Cells Induces Fas-mediated Apoptosis. M. H. Lareef, Q. Tahin, I. H. Russo, G. Mor, J. Song, D. Mihaila, C. M. Slater, and J. Russo. *Fox Chase Cancer Center, Philadelphia, PA, and Yale University, New Haven, CT.*

The human breast epithelial cells (HBEC) BP1E, which have been transformed with the chemical carcinogen benz(a)pyrene (BP), express *in vitro* phenotypes indicative of neoplastic transformation and microsatellite instability at 17p13.1. Transfection of chromosome (chr) 17 to BP1E cells inhibits the transformation phenotypes, an indication that the transfected 17p13.1 region reverts the neoplastic process. This work was designed for determining whether the reversion was mediated by activation of Fas receptors, which are known to induce apoptosis. We used MCF-10F, BP1E, BP1E-11neo, BP1E-17neo, and BP1E-14hygro cell lines for testing their sensitivity to Fas-induced apoptosis. The cells were incubated in triplicate with anti-human Fas monoclonal antibody at the concentrations of 50, 100, 150, and 200ng/ml for 24 hr, and then with MTT; absorbency was measured at 540nm for determination of the percentage of absorbency relative to the control normal mouse IgG antibody. MCF-10F cells and 7 out of 10 BP1E-17neo clones exhibited a dose dependent maximal sensitivity. BP1E cells, BP1E-11neo, BP1E-14hygro, and 3 BP1E-17neo clones were resistant at all doses tested. PCR analysis revealed that the 7 sensitive clones contained the 17p13.1 region (markers D17S1852, D17S796, D17S13 and TP53), which was absent in 3 resistant clones. Our data indicate that the reversion of the transformed phenotype by chr17 transfection is mediated by FAS receptor activation. (Supported by NCI Grant R01 CA67238 and DAMD 17-00-1-0249).

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92nd Annual Meeting March 24-26, 2001 New Orleans, LA.

#4781 RNA Relocation at Mitosis in Benzo α Pyrene-Transformed Human Breast Epithelial Cells after Microcell-Mediated Transfer of Chromosomes 11 and 17. Maria Luiza Silveira Mello, M. T M H Lareef, Y. F. Hu, X. Q. Yang, Benedicto C. Vidal, and J. Russo. *Fox Chase Cancer Center, Philadelphia, PA, and UNICAMP, Campinas, Brazil.*

RNA relocation in association with mitotic spindle fibers does not vary during cell division in the benzo α pyrene (BP)-transformed MCF-10F cell series. However, the frequency of RNA-containing nucleolus-like bodies persistent during mitosis decreases severely in the transformed and tumorigenic MCF-10F cells in comparison with the non-transformed cells. This has been associated with an improved use of RNA transcripts with cell transformation and tumorigenesis at least under in vitro conditions. Here, RNA relocation and incidence of nucleolus-like bodies were studied during mitosis in tumorigenic BP-transformed MCF-10F cells (BP1-E1) subjected to the microcell-mediated transfer of normal chromosomes 11 and 17. In all cases the RNA relocation associated with the mitotic spindle did not vary. The frequency of the nucleolus-like bodies, which in BP1-E1 cells was < 15% increased to 74% and 19.5% with the introduction into the BP1-E1 cells of the chromosomes 11 and 17, respectively. The frequency of the nucleolus-like bodies in the cells with chromosome 11 insertion came closer to that of the non-transformed MCF-10F cells (86%). Although published data reveal that the insertion of chromosome 11 into BP1-E1 cells inhibited cell growth and reduced colony size only partly and less efficiently than the insertion of chromosome 17 did, the chromosome 11 is suggested to play a significant role in the control of the fate of the RNA transcript production. (Supported by FAPESP, CNPq, and NIH grant RO1 CA67238)

Reprinted from Proceedings of American Association for Cancer Research
92nd Annual Meeting March 24-26, 2001 New Orleans, LA.

#4803 Chromosome 17 (p13.1) Transfer Reverts Transformation Phenotypes in Human Breast Epithelial Cells. M. H. Lareef, Q. Tahin, I. H. Russo, D. Mihaila, J. Tomaz, A. Tosolini, J. Testa, and J. Russo, *Fox Chase Cancer Center, Philadelphia, PA.*

BP1E cells, derived from benz (a) pyrene (BP) transformed MCF-10F cells, form colonies in agar methocel, having high colony efficiency (CE), lack ductulogenic capacity in collagen, are tumorigenic in SCID mice, and express genomic alterations in chromosomes (chr) 11 and chr17. In order to test whether one or both chromosomes play a functional role in the expression of transformation phenotypes we transfected BP1E cells with chr11 or chr17 by microcell mediated chromosome transfer (MMCT). After G418 selection, 16 primary microcell hybrids were cloned from chr11 and 19 from chr17 transfected cells and designated BP1E-11neo and BP1E-17neo, respectively. Ten clones were randomly selected from each one of them and tested for the presence of the donor chromosome by fluorescent *in situ* hybridization (FISH) and polymerase chain reaction-based restriction fragment-length polymorphism analyses. For identifying the locus transferred 44 markers were utilized for chr 17 and 45 for chr11. Seven BP1E-17neo clones were similar to MCF-10F cells in morphology, population doubling time, ductulogenic capacity, and did not form colonies. Three clones had High CE and lost ductulogenic capacity. PCR analysis revealed that the 7 clones similar to MCF-10F cells contained the p13.1 region, confirmed by markers D17S1852, D17S796, D17S513, and TP53. BP1E-11neo clones, like the parental BP1E cells, had high CE and were not ductulogenic. These results indicate that the expression of transformation phenotypes is under the control of the genes harbored in the 17p13.1 region. (Supported by NCI grant R01 CA67238 and DAMD17-00-1-0249)

Reprinted from Proceedings of 14th International Congress of Cytology-
May 27-31, 2001 RAI Congress Center-Amsterdam- The Netherlands.

456: CHANGES IN CHROMATIN TEXTURE IN TRANSFORMED CELLS AS ASSESSED BY MOLECULAR BIOLOGY ASSAYS AND IMAGE ANALYSIS

M.L.S. Mello¹, B. de Campos Vidal¹, M.T. Lareef², J. Russo². (1) Institute of Biology, UNICAMP, Campinas, SP, Brazil. (2) Fox Chase Cancer Center, Philadelphia, PA, USA.

Molecular changes associated with breast cancer progression have been characterized using the MCF-10F cell series. The MCF-10F cell line was originally established from fibrous mastectomy tissue of a patient without detectable cancer. When MCF-10F cells were treated by the carcinogen benzo [a] pyrene (BP), different cell lines with gradual steps of tumoral progression (BP1, BP1-E, BP1-E1, BP1-Tras, among others) have been produced. We have been searching relationships amidst changes in DNA content, interphase cell chromatin supraorganization (defined in terms of chromatin texture), and the expression of different stages of tumorigenesis in these cells. Image analysis studies have revealed decreased nuclear areas simultaneous to an increase in chromatin higher order packing states and nucleolar areas and to DNA losses accompanying the expression of the in vitro tumorigenesis stages established in the MCF-10F cell series by J. Russo and his co-workers, with molecular biology assays. Since affected chromosomes 11 and 17 have been associated with the neoplastic progression in BP-transformed MCF-10F cells, image analysis of Feulgen-stained tumorigenic BP1-E cells subjected to microcell-mediated chromosome transfer involving unaffected chromosomes 11 and 17 was performed. The results indicated reversal of DNA amounts and nuclear sizes to a state typical of non-transformed cells, especially after the transfer of chromosome 17, whereas no reversal in chromatin texture was elicited. While chromosome 17 revealed to be primordial for normality recovery, a more complex return to genome balance is probably required for the whole chromatin to recover its normal supraorganization. (Support: FAPESP/99/2547-8, CNPq/460621/2000-9, NIH RO1CA67238)

Seminar XI: DNA, Flow and Image Cytometry: May 30: 10.00 - 12.00: Oral presentation: 15 min.

RNA relocation and nucleolus-like bodies persistence at mitosis in benzo[a]pyrene-transformed human breast epithelial cells after microcell-mediated transfer of chromosomes 11 and 17

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Running title: RNA relocation and nucleolus-like bodies persistence

The fate of presumed nucleolar RNA accumulated during mitosis was studied cytochemically (critical electrolyte concentration assay variant) in benzo[a]pyrene (BP)-transformed human breast epithelial MCF-10F cells after microcell-mediated transfer of normal 11 and 17 chromosomes. The aim was to detect changes induced by insertion of unaffected 11 and 17 chromosomes to tumorigenic MCF-10F cells (BP1-E cell line), since alterations in these chromosomes are involved in the expression of the transformed phenotype in MCF-10F cells, and tumorigenic MCF-10F cells exhibit much fewer nucleolus-like bodies than non-transformed MCF-10F cells. The pattern of RNA relocation in association with the mitotic spindle was not found to vary in the cell lines analyzed. With the introduction of chromosome 17 in BP1-E cells, the frequency of persistent nucleolus-like bodies decreased or was practically unaffected. However, in cells with transferred normal chromosome 11 the frequency of nucleolus-like bodies came closer to that of non-transformed MCF-10F cells. It is suggested that normal chromosome 11 (in contrast to chromosome 17) contributes to maintenance of the RNA surplus which accumulates in nucleolus-like bodies during cell division of the human breast epithelial cells at least *in vitro*. With gradual steps of tumoral progression in transformed MCF-10F cells, alterations in chromosome 11 may lead cells to an improved use of their RNA transcripts.

Key words: RNA, nucleolus-like bodies, human breast epithelial cells, microcell-mediated chromosome transfer, chromosome 11, chromosome 17

1. Introduction

Human breast epithelial cells (MCF-10F) when transformed with benzo[a]pyrene (BP) produced different cell lines with gradual steps of tumoral progression (BP1, BP1-E, BP1-E1 among others) [2].

RNA relocation in association with mitotic spindle fibers as demonstrated cytochemically with a critical electrolyte concentration assay variant [4] does not vary during cell division in the BP-transformed MCF-10F cell series [6]. However, the frequency of RNA-containing nucleolus-like bodies persistent during mitosis decreases drastically in the transformed and tumorigenic MCF-10F cells in comparison with the non-transformed cells [6]. On the other hand, a significant increase in nucleolar sizes and rRNA production in interphase nuclei was revealed in the BP1-E and BP1-E1 tumorigenic cell lines in comparison with non-tumorigenic, transformed cell line BP1 and non-transformed MCF-10F cells [1]. These findings have been associated with an improved use of RNA transcripts with cell transformation and tumorigenesis, at least under *in vitro* conditions [6].

Microsatellite instability in chromosomes 11 and 17 [3] and loss of heterozygosity in chromosome-17 [7] among alterations in some other chromosomes have been associated with the neoplastic progression in BP-transformed MCF-10F cells. Microcell-mediated chromosome transfer assays have demonstrated that specific regions of chromosomes 11 and 17 play a functional role in the expression of the transformed phenotype in MCF-10F cells [8]. Considering that the insertion of normal chromosomes 11 and 17 to the tumorigenic human breast epithelial cells could also

affect their RNA distribution pattern during cell division, RNA relocation and incidence of nucleolus-like bodies were studied during mitosis in tumorigenic BP-transformed MCF-10F cells subjected to the microcell-mediated transfer technique involving normal chromosomes 11 and 17.

2. Materials and methods

2.1. Cells

BP1-E, a BP-transformed cell line derived from MCF-10F cells currently maintained in the Breast Cancer Research Laboratory of the Fox Chase Cancer Center at Philadelphia under conditions reported previously [2], was subjected to transfer of normal chromosomes 11 and 17 by microcell-mediated chromosome transfer (MMCT). BP1-E cells were transfected with pSV2neo plasmid using the Calphos Maximizer Transfection Protocol (Clontech, Palo Alto, CA) and then fused with microcells generated from human chromosome donor cells, A9-11neo or A9-17neo, giving rise to microcell hybrids, BP1E-11neo and BP1E-17neo, respectively. Colonies surviving in DMEM medium containing G-418 (400 µg/ml) were subcloned. Four expandable clones (subclones) containing normal chromosomes 11 and 17 were used. BP1-E cells at passage 45, and BP1E-11neo cells and BP1E-17neo cells at passage 7 were used. The cells were grown for 48 h (BP1-E, BP1E-11neo and BP1E-17neo) and 96 h (BP1E-17neo) on well slides and fixed. Since BP1-E cells and the clone with transferred chromosome 11 grew faster than the clone transferred with chromosome 17, the

confluence value of 80% was attained by these cells at different growth times (BP1-E and BP1E-11neo, 48 h; BP1E-17neo, 96 h).

2.2. Cell preparations and staining procedure

The cells were fixed in an ethanol-acetic acid mixture(3:1, v/v) for 1 min, rinsed in 70% ethanol for 3-5 min, and air dried. Then they were subjected to the critical electrolyte concentration (CEC) assay variant for the identification of RNA, a method that uses toluidine blue (TB) and Mg^{2+} ions as competitors for the substrate binding sites. At the DNA CEC point, while DNA metachromasy (violet colour) is abolished, RNA metachromasy remains unchanged ($CEC_{RNA} > CEC_{DNA}$) [5]. Briefly, the cells were stained with a 0.025% TB (Merck) solution in McIlvaine buffer at pH 4.1 for 15 min and then treated with a 0.05 M aqueous $MgCl_2$ solution for 15 min, rinsed in distilled water, air-dried, cleared in xylene and mounted in Canada balsam [5]. Preparations treated with a 0.01% RNase III (Sigma) aqueous solution at 37°C for 1 h prior to the CEC assay were used as a control. Observations and photomicrographs were carried out with a Zeiss Axiophot II microscope.

2.3. Mitosis counting

Three slides (total: six wells) of each cell line (or clone) were examined for mitotic cells. Nearly 70 dividing cells were chosen at random per slide and classified for presence or absence of nucleolus-like bodies, with the exception of the analysis of mitosis in BP1E-17neo cells grown for 48 h, when the number of dividing cells considered per well was equal to 25.

3. Results and Discussion

RNA was identified in the preparations subjected to the CEC assay variant by the metachromatic staining which can additionally be prevented by previous treatment with RNase [5]. The metachromatic staining appeared concentrated in the nucleoli of the interphase cell nuclei (Figs. 1a, b) or lined finely the chromosomal mass migrating to equatorial plate of the mitotic cells (Figs. 1a, b, e, f) and then interspaced the sets of chromosomes moving to the cell poles during anaphase/telophase (Figs. 1g, h). In all cases analyzed here, the pattern of RNA relocation associated with the mitotic spindle was found to be the same and agrees with previous data [6].

When nucleolus-like metachromatic bodies were present, they appeared close to the chromosomes or the mitotic spindle in the dividing cells (Figs. 1a-d, f, g). The frequency of the nucleolus-like bodies in mitotic cells of the various cell lines is reported in Table 1. The results indicate that with the introduction into the BP1-E cells of chromosome 17, the frequency of nucleolus-like bodies was small, even when confluence of the cell culture was the same as that of cultures of BP1-E cells with transferred chromosome 11. Only in cells with transferred chromosome 11 the frequency of the nucleolus-like bodies came closer to that of the non-transformed MCF-10F cells (86%) [6].

Published data reveal that the insertion of normal chromosome 11 into BP1-E cells inhibits cell growth and reduces colony size only partly and less efficiently than the insertion of normal chromosome 17 does [8]. Indeed, it was found here that BP1E-

11neo cells grew faster than BP1E-17neo cells. The normal chromosome 11 did not slow significantly the cell growth dynamics as chromosome 17 did.

However, the larger frequency of the nucleolus-like bodies verified in the BP1E-11neo cells may reflect a certain miscontrolled RNA transcript production, at least *in vitro*, making that a certain RNA surplus was not consumed and became accumulated in nucleolus-like bodies during cell division, much resembling in this aspect non-transformed MCF-10F cells [6]. Presence of normal chromosome 11 contributes to maintenance of RNA surplus during cell division in this cellular system *in vitro*. On the other hand, the cells with transferred normal chromosome 17 but not of normal chromosome 11, which grew slower and only slightly presented increase in their frequency of nucleolus-like bodies may continueing make a better use of their RNA transcripts like tumorigenic BP1-E cells do or their RNA production requirements decrease in presence of normal chromosome 17 specifically, although alterations in other chromosomes may still be present [3].

Acknowledgments

This investigation was supported by the State of São Paulo Research Support Foundation (FAPESP, grant no. 99/02547-8) and by NIH grant RO1 CA67238. MLSM and BCV were the recipients of fellowships and grant no. 460621/2000-9 from the Brazilian National Council for Research and Development (CNPq).

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Figure Legends

Fig. 1. Metachromatic staining in interphase cell nucleoli (nu)(a, b), nucleolus-like bodies (arrows)(a-d, f, g), and RNA along the mitotic spindle (a, g, h) in MCF-10F cell lines. E, h: cells devoid of conspicuous nucleolus-like bodies; ch: chromosomes. a-d, g, h, x1800; e, f, x350

Table 1

Incidence of nucleolus-like bodies

Cells	Cell culture		Metaphases	
	Growth (h)	Confluence (%)	no. of cells counted	nucleolus-like bodies frequency (%)
BP1-E	48	80	218	16
BP1E-11neo	48	80	221	74
BP1E-17neo	48	50	150	6.7
	96	80	246	19.5

8:00 AM - 12:00 PM

4781 RNA Relocation at Mitosis in Benzo[A]Pyrene-Transformed Human Breast Epithelial Cells after Microcell-Mediated Transfer of Chromosomes 11 and 17

Maria Luiza Silveira Mello, M. T M H Lareef, Y. F. Hu, X. Q. Yang, Benedicto C. Vidal, J. Russo, UNICAMP, Campinas, Brazil; Fox Chase Cancer Center, Philadelphia, PA.

8:00 AM - 12:00 PM

4782 Anti-Apoptotic Role of IL-6 in Human Cervical Cancer Is Mediated by PI 3K/Akt-Dependent Up-Regulation of Mcl-1

Lin-Hung Wei, Min-Liang Kuo, Chang-Yao Hsieh, National Taiwan University Hospital, Taipei, Taiwan.

8:00 AM - 12:00 PM

4783 Effects of Whey Protein Consumption on Initiation and Promotion of DMBA-Induced Mammary Tumors in Rats

Reza Hakkak, Soheila Korourian, Martin J.J. Ronis, J. Craig Rowlands, Thomas M. Badger, Arkansas Children's Nutrition Center, Departments of Pediatrics and Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.

8:00 AM - 12:00 PM

4784 S100p Is a Marker of Cell Immortalization, Preceding Phenotypic Expression of Neoplastic Transformation in Human Breast Epithelial Cells

C. M. Slater, M. H. Lareef, I. H. Russo, J. Tomaz, V. Band, J. Russo, Fox Chase Cancer Center, Philadelphia, PA; New England Medical Center, Boston, MA.

8:00 AM - 12:00 PM

4785 Mutagenicity of 2-Amino-1-Methyl-6-Phenylimidazo [4,5-B] Pyridine (PhIP) in the Mouse Mammary Gland in Organ Culture

Laundette P. Knight Jones, Mau Tran, Herman A.J. Schut, Elizabeth G. Snyderwine, National Cancer Institute, NIH, Bethesda, MD; Medical College of Ohio, Toledo, OH.

8:00 AM - 12:00 PM

4786 Comparative Mammary Carcinogenicity in Female CD Rats of a Bay Region Diol Epoxide and a Fjord Region Diol Epoxide, Metabolites of the Environmental Contaminant Benzo[c]Chrysene

J. M. Lin, D. Dessai, K. El-Bayoumy, S. Amin, American Health Foundation, Valhalla, NY.

8:00 AM - 12:00 PM

4787 Reduced Caloric Intake Resulting from Weight-Cycling Decreases Incidence and Increases Latency of Mammary Tumor Development in MMTV-TGF- α /Lep⁺Lep^{ob} Female Mice to a Greater Extent than Does Chronic Food Restriction

Margot P. Cleary, Michelle K. Jacobson, Frederick C. Phillips, Susan C. Getzin, Nita J. Maihle, Joseph P. Grande, University of Minnesota, Austin, MN; Mayo Clinic, Rochester, MN.

8:00 AM - 12:00 PM

4788 The Microsomal Anti-Estrogen Binding Site (AEBS) is a Multiproteic Complex Involved in the Metabolism of Cholesterol

Blandine Kedjouar, Philippe DeMedina, Jean-Charles Faye, Marc Etienne Poirot, Inserm U 397, Toulouse, France.

8:00 AM - 12:00 PM

4789 Biological and Molecular Mechanism of the Progesterone Receptor Antagonist, Onapristone

Simon Jonathan Crook, David Brook, John F.R Robertson, University of Nottingham, Nottingham, UK; City Hospital, Nottingham, UK.

8:00 AM - 12:00 PM

4790 Cytogenetic Findings in Ovarian Clear Cell Carcinoma Detected by Comparative Genomic Hybridization and Microsatellite Analysis

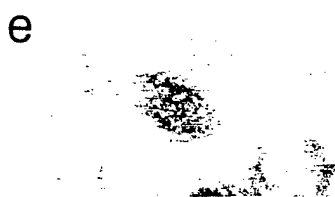
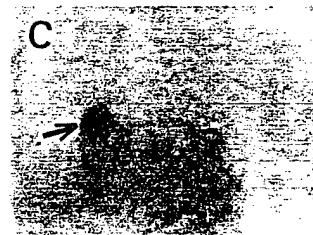
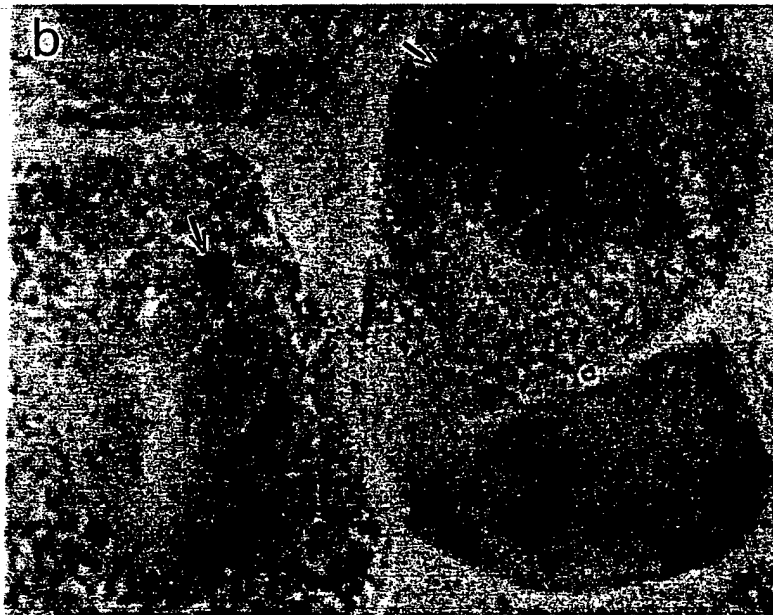
Jo Dent, T. Perren, N. Wilkinson, I. Richmond, A. Markham, S. Bell, St James's Hospital, Leeds, UK; Hull Royal Infirmary, Hull, UK; University of Leeds, Leeds, UK.

8:00 AM - 12:00 PM

4791 Estrogen-Induced Iron Accumulation in Human Mammary Cell Lines

J Shawn Jones, Charlotte H. Clarke, Mary L. Thomas, Joachim G. Liehr, University of Texas Medical Branch, Galveston, TX; CIPHERGEN Biosystems, Inc., Fremont, CA; Stehlin Foundation, Houston, TX.

8:00 AM - 12:00 PM



Coping Style Correlates of Participation in Genetic Testing for Inherited Breast and Ovarian Cancer Risk

Suzanne M. Miller, Ph.D., Jennifer L. Driscoll, M.S., Michelle Rodoletz, Ph.D., Kerry A. Sherman, Ph.D., Mary B. Daly, M.D., Ph.D., Michael Diefenbach, Ph.D., Joanne S. Buzaglo, Ph.D., Andrew K. Godwin, Ph.D., & James S. Babb, Ph.D., Fox Chase Cancer Center, Philadelphia, PA.

This study focused on the psychosocial factors involved in decision-making for BRCA genetic testing among women ($N = 281$) undergoing genetic counseling because of a putative hereditary family history of breast and/or ovarian cancer. We explored whether high monitors (who scan for and amplify disease-related threats) differed in their cognitive and affective reactions to testing in comparison with low monitors (who typically distract from threat-related cues). Participants attended an initial group breast and ovarian cancer educational session, followed by an individualized cancer-risk counseling session. Psychosocial assessments were completed before the group educational session, before individualized risk counseling, and 1 week following the decision to donate blood for genetic testing. Outcomes of interest included satisfaction with the decision to donate blood and disease-related intrusive and avoidant ideation, as measured by the Revised Impact of Events Scale (RIES). The majority of the sample (60%) were unaffected relatives and well-educated, with 46% having at least a college education and 21% having graduate degrees. The average age was 47 years ($SD = 12$). The results indicated that there was no significant difference in the decision to donate blood based on attentional style, with 95% of women providing a blood sample. However, there were significant differences between high and low monitors on measures of psychosocial adjustment 1 week post-blood draw. Specifically, high monitors were more satisfied with their decision to donate blood than low monitors ($p < .05$). Nonetheless, high monitors displayed greater disease-specific intrusive ($p < .05$) and avoidant ($p < .01$) ideation than did low monitors. These results suggest that dispositional attentional style is an important factor when designing and tailoring programs for genetic testing outreach and education. Ongoing research seeks to extend these findings to a sample of low income, minority women.

Poster presented at A Decade of ELSI Research, January 16-18, 2001, Bethesda, MD.

PREDICTORS OF BREAST SELF-EXAMINATION FREQUENCY IN YOUNG WOMEN

Kerry A. Sherman, Ph.D., Fox Chase Cancer Center; Natalie Mutton, B.A., Cindy Nour, B.A., Alisa de Torres, M.A., Sarah Wainwright, B.A., Macquarie University, Sydney.

Breast self-examination (BSE) is a convenient and easily performed measure for the early detection of breast cancer, yet the majority of women are not practicing BSE. For younger women, BSE is especially important, since they fall below the recommended age for mammographic screening. Guided by the Cognitive-Social Health Information Processing framework (Miller et al., 1996), the objective of this study was to examine psychosocial factors associated with BSE frequency in young Australian women (18-39 years), since little is known about breast health practices of this age group. A cross-sectional design was employed utilizing a community sample of 181 women (mean age 26.2; 84% White-Australian, 9% Asian-Australian, and 7% African/Middle Eastern-Australian); the majority (95%) described their current health status as average or better than average. Participants completed a self-administered questionnaire measuring background variables (age, family history of breast cancer), cognitive variables (perceived vulnerability, breast cancer knowledge), and affective variables (cancer worry and general distress). General distress was measured using the anxiety subscale of Depression Anxiety Stress Scales (Lovibond & Lovibond, 1995). Multiple stepwise regression analyses controlling for family history indicated that familiarity with BSE, breast-cancer specific worry, and age were significant predictors of BSE frequency ($p < .001$). Increased levels of familiarity, worry and age were associated with greater BSE frequency. The results have implications for the design of educational messages that acknowledge feelings of cancer-specific worry and provide guidance in BSE technique and health protective behaviors for younger women.

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Rapid communications poster presented at Society of Behavioral Medicine 22nd Annual Scientific Session, March 21-24, Seattle, WA.

MONITORING STYLE IN LOW-INCOME MINORITY WOMEN AT RISK FOR CERVICAL CANCER: COGNITIVE SOCIAL DETERMINANTS OF ADJUSTMENT AND ADHERENCE

Suzanne M. Miller, Ph.D., Michelle Rodoletz, Ph.D., Joanne Buzaglo, Ph.D., Terri Gray, B.S., & Sherman, K.A., Fox Chase Cancer Center

Early detection and management of pre-cancerous cervical disease can prevent the development of invasive cervical carcinoma. Nevertheless, mortality rates among minorities remain high, largely due to non-adherence with medical recommendations. This study explored the moderating role of cognitive coping style (high vs. low monitoring) in promoting adherence and adjustment to cervical cancer risk in response to two preparatory counseling interventions. Low-income, minority women (N=94; 88% African-American, 9% Hispanic; mean age 27) received either two sessions of Stress Inoculation Training (SIT), two sessions of Health Education (HE), or usual care (UC) prior to their initial diagnostic colposcopy following an abnormal test result. Patients provided background demographic and medical information and completed the Monitor Blunter Style Scale at baseline. The Revised Impact of Events Scale, the Spielberger Anxiety Scale, and the CES-D were assessed at baseline and post-intervention. Medical charts were reviewed at 15-months post-colposcopy to ascertain long-term adherence with medical recommendations. Analyses of variance controlling for age, education, and baseline depression and anxiety found high monitors displayed the greatest reduction in disease-specific avoidant ideation ($p<.01$) in the SIT and HE conditions. Conversely, low monitors fared better with respect to avoidant ($p<.01$), as well as intrusive ideation ($p<.05$), within UC. A logistic regression controlling for relevant mediators found intrusive ideation to be a significant predictor of adherence with long-term (1-year) regimens ($p<.01$). The findings suggest that tailoring psycho-educational interventions to cognitive coping style may enhance adjustment, and thereby adherence, among at-risk inner-city populations.

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CCG EMPIRICAL ABSTRACT

Total # of words = 298

Part of Symposium Presentation and Paper, Society of Behavioral Medicine 22nd Annual Scientific Session, March 21-24, Seattle, WA.

MONITORING-BLUNTING BEHAVIORAL SIGNATURES IN COPING WITH HEALTH-RELATED THREATS: THE
EXAMPLE OF CANCER RISK AND DISEASE

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In press: Psicologia della Salute

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Over the past decade, health psychology research has successfully applied basic psychological concepts to furthering our understanding of the role of individual differences in dealing with health-related challenges and stressors (e.g., Lazarus, 1991; Rothman & Salovey, 1997; Taylor, 1990). In this paper, we will apply to health psychology a comprehensive theoretical framework, the “cognitive-affective” approach to individual differences (Miller, Shoda & Hurley, 1996), that integrates cumulative findings and theorizing from diverse areas of cognitive-behavioral science and evidence-based counseling and communication interventions (e.g., Carver & Scheier, 1981; Sarason, 1979). First, we provide an overview of our cognitive-affective framework, which delineates how individuals cognitively and affectively process information about health threats (Miller & Schnoll, 2000). In particular, we highlight two distinctive cognitive-affective signatures (“monitoring” versus “blunting”) in response to health threats, using the cancer context as an exemplar. Cancer provides an ideal paradigm for studying the response to health-related challenges, since cancer-related threats are often probabilistic and complex. Hence, they require sustained decision making, adherence, and modulation of anxiety over time (Miller & Diefenbach, 1998). Further, cancer spans the spectrum of healthy individuals with average cancer risk, to individuals with high risk (e.g., due to genetic predisposition, prior exposure to carcinogens, or socio-demographic disadvantage), to individuals already diagnosed with cancer, to cancer survivors.

Cognitive-Affective Processing of Cancer Risk and Disease Information

The recently developed Cognitive-Social Health Information Processing (C-SHIP) model provides an overarching framework that identifies the basic cognitive-affective processes that underlie how individuals encode, perceive, and react emotionally and behaviorally to potentially stressful health-related information (Miller, Shoda, & Hurley, 1996; Miller, Fang, Diefenbach, & Bales, in press). This approach highlights five major cognitive-affective (C-SHIP) mediating units that are activated when confronting health threats and that determine adaptive behavior not only in the short term, but also over time (Leventhal, Diefenbach, & Leventhal, 1992; Miller, Shoda, & Hurley, 1996). These mediating units include individuals' encodings and self-construals, their cancer-related expectancies and beliefs, their affects and emotions, their health-related values and goals, and their self-regulatory coping strategies (Miller, Shoda & Hurley, 1996). From the C-SHIP perspective, individuals differ in their prototypic, or signature, responses to health-relevant information in two main ways: the ease of activation of the mediating units and the structure of relations among the mediating units, once activated (Miller, Shoda, & Hurley, 1996). For example, in terms of ease of activation, learning that a close relative has been diagnosed with breast cancer may heighten perceived vulnerability to cancer for one individual, whereas, for another individual, this information may

Monitoring and Health Threats have no effect on perceived vulnerability. In terms of the pattern of activation among the mediating units, perceived vulnerability may trigger the expectancy that the individual will develop breast cancer and the belief that there is nothing she can do to lower her breast cancer risk, thereby increasing her distress. However, in contrast, another individual who feels equally vulnerable to the disease may believe that if she follows recommended screening regimens, she can detect disease at an early stage, thereby reducing her distress.

Monitor-Blunter Behavioral Signatures in Response to Health Threats

In our research, we have focused on two key prototypic, or "signature" information-processing styles in response to health-related threats, "monitoring" and "blunting" (Miller & Diefenbach, 1998). Monitors generally scan for, and magnify, threatening health-related cues; whereas blunters distract from, and downgrade, threatening information (Miller, 1996). The cognitive-affective and behavioral consequences of these signature styles have been explored in a variety of health-risk contexts, in particular, in response to cancer risk feedback, prevention, and management. These signature responses have been found to predict individual differences in cognitive-affective responses and coping behaviors to health-related stressors. The Monitor-Blunter Style Scale (MBSS) was developed and validated to distinguish between "monitors" and "blunters" (Miller, 1987). The scale has been translated into a number of languages and used in a variety of cancer-related settings, with populations such as gynecologic patients with precancerous cervical disease (e.g., Miller, Buzaglo, et al., 1999; Miller, Roussi, et al., 1994), women at risk for breast and ovarian cancer (e.g., Basen-Engquist, 1997; Diefenbach, Miller, & Daly, 1999; Lerman, Daly, Masny, & Balshem, 1994), patients with cancer (e.g., Gard, Edwards, Harris, & McCormick, 1988; Lerman et al., 1990), and healthy women undertaking self-screening cancer regimens (Jacob, Penn, Kulik, & Spieth, 1992). It has also been used in a number of other threatening medical contexts and populations (e.g., Litt, Nye, & Shafer, 1995; Miller, Mischel, Schroeder, et al., 1996; Phipps & Srivastava, 1997; Steptoe & O'Sullivan, 1986). We now systematically review the literature on monitoring and blunting with respect to each of the five cognitive-affective mediating units.

Encodings and Self-Construals. As individuals are confronted with new or unfamiliar health-relevant feedback, they need to accommodate and assimilate this information to their existing health-related schemas (Miller, Fang, Diefenbach, & Bales, in press). Encodings refer to the individual's mental representations about the self and the health threat situations (Miller, Shoda, & Hurley, 1996). Health-relevant encodings and self-construals refer to how the individual evaluates incoming threat and disease-relevant information (e.g., genetic risk feedback, disease prognosis, treatment recommendations). For example, when a woman with a personal, or family, history of breast cancer detects a breast lump, the experience is likely to trigger memories of breast disease; whereas a woman with

no history of breast disease is likely to appraise the lump as benign (Miller, Shoda, & Hurley, 1996). Individual differences in encoding influence the relationship between the individual's objective health status and his or her perceived susceptibility to disease (Miller, Brody, & Summerton, 1988; Muris & van Zuuren, 1992). Since monitors seek out and focus on threatening cues, they are more likely than blunters to develop threat-laden encodings when encountering stressful health situations. For example, in a study of first-degree relatives (FDRs) of ovarian cancer patients, monitors perceived themselves to be at greater risk for developing the disease than blunters, independent of true levels of risk (Schwartz, Lerman, Miller, Daly & Masny, 1995; Fang, Miller, et al., in press). Hence, their construals of personal cancer risk are characterized by elevated perceptions of vulnerability (Schwartz et al., 1995), which can ultimately undermine adaptive health-protective behaviors (Kash et al., 1992; Lerman, Daly, Sands, et al., 1993).

Expectancies and Beliefs. How an event is perceived and formed in memory can generate expectancies and beliefs about what is likely to happen (Miller, Green, & Bales, 1999). Expectancies encompass the individual's self-efficacy beliefs (e.g., "I can perform regular breast self-examination"), and the anticipated outcomes of particular courses of action (e.g., "I can reduce the likelihood of developing breast cancer if I perform regular breast self-examination") (Bandura, 1986; Lau, Bernard, & Hartman, 1989; Leventhal, Diefenbach, & Leventhal, 1992). These expectancies can significantly affect subsequent health behaviors and emotional responses (Carver et al., 1993; Scheier & Carver, 1985; Stefanek & Wilcox, 1991). Research has shown that monitors and blunters are characterized by distinctive expectations and beliefs about the type and outcomes of health threats, with monitors more likely to have negative expectations about the severity and consequences of health threats. For example, in a study of women undergoing diagnostic follow-up for an abnormal Pap smear, monitors perceived their condition to be more serious than blunters, and were more likely to blame themselves for their health problem (Miller et al., 1994). Monitors are also more likely than blunters to believe that they will experience distress in response to cancer threats, such as genetic testing feedback for women at risk for breast-ovarian cancer (Lerman, Daly, Masny, & Balshem, 1994). In summary, since monitors are more likely to amplify the threatening aspects of cancer-relevant cues and experiences, this focus on threat can promote negative expectations about their condition, its consequences, and their beliefs about their ability to cope with the perceived cancer threat.

Affect. Individuals vary in their affective responses to health-related threats (Horowitz, 1991; Leventhal, Diefenbach, & Leventhal, 1992), especially with respect to their levels of disease-specific worry (Lerman, Kash et al., 1994) and intrusive ideation (Lerman, Daly, Sands et al., 1993; Lerman, Miller et al., 1991; Lerman, Trock et al., 1991). Monitoring-blunting signature styles have been found to influence the extent and intensity of distress

experienced by individuals across the disease spectrum. For example, among women at familial risk for ovarian cancer, undergoing ultrasound scan, monitors reported greater distress in response to receiving a false positive result than did blunters, both immediately, and at one-year follow-up (Wardle, 1995). In a study of decision making among women at risk for ovarian cancer, monitors tended to feel more vulnerable to cancer and more distressed and anxious about their cancer risk, compared to blunters (Fang, Miller, Daly, & Hurley, in press). Similarly, among cancer patients undergoing chemotherapy, monitors were more likely than blunters to experience anxiety prior to treatment and to report higher levels of depression during the administration of chemotherapy (Lerman et al., 1990), as well as greater and more prolonged nausea and vomiting (Gard et al., 1986). Monitors are also more likely to experience sustained discomfort in response to diagnostic and treatment procedures than are blunters (Miller & Mangan, 1983; Miller, Roussi et al., 1994). Taken together, these findings suggest that monitors are particularly vulnerable to high levels of distress and intrusive ideation when faced with health threats. This heightened sense of vulnerability and distress can lead to avoidant ideation and behaviors, thereby undermining adherence and effective coping (Kash et al., 1992; Lerman, Daly, Sands, et al., 1993; Miller, Rodolitz et al., 1996), and interfering with rational decision-making processes (Miller, Fang, et al., 1999).

Values and Goals. Values concern the degree of importance that individuals assign to their health. Values, in turn, shape an individual's goals with respect to health-relevant feedback and management recommendations (Miller, Shoda, & Hurley, 1996). An important health value/goal has to do with the individual's desire for information and level of involvement in health care, some patients want to be informed about their cancer risk (e.g., Croyle & Lerman, 1993; Lerman, Daly, Masny, & Balshem, 1994) and assume an active role in decision-making (e.g., Cassileth, Zupkis, Sutton-Smith, & March, 1980), while other patients prefer to remain more uninformed (e.g., Jones, 1981) and to take a more passive role (e.g., Degner & Sloan, 1992). Monitoring and blunting styles influence the individual's health-relevant values and goals: while monitors desire and seek out extensive information about their health status, blunters prefer to obtain less detailed information (Lerman, Daly, Walsh, et al., 1993; Miller, 1987; Steptoe, Sutcliffe, Allen, & Coombes, 1991). For example, a study of the information-seeking behaviors of adult daughters of women with early breast cancer found that monitors sought more information from cancer-related organizations and newspapers than blunters did (Rees & Bath, 2000). Given the high need for detailed information, monitors tend to be less satisfied with the amount of information received in standard care (Miller & Mangan, 1983; Steptoe & O'Sullivan, 1986). In the oncologic context, studies of early stage breast cancer patients (Lerman, Daly, Walsh, et al., 1993) and metastatic cancer patients (Steptoe et al., 1991) show that monitors are less satisfied with physician communications than blunters. Monitors are also generally more

demanding than blunters are, placing a high level of importance on kindness and reassurance from their physicians, and desiring more medical interventions, such as diagnostic tests and prescriptions (Miller, Brody, & Summerton, 1988).

Self-Regulatory and Coping Strategies. To sustain adherence to health-protective practices and treatment regimens, particularly over time, individuals must be able to plan effectively (e.g., developing a strategy to remind themselves to perform breast self-examinations) and successfully manage interfering health-related distress (Carver, et al., 1989; Miller, Mischel, O'Leary, & Mills, 1996; Miller, Shoda, & Hurley, 1996). Under conditions of relatively low threat, such as routine health screening, monitors are more likely than blunters to schedule and adhere to recommended regimens (e.g., mammography, Pap smears, and colorectal exams) (Christensen, Smith, Turner, & Cundick, 1994; Steptoe & O'Sullivan, 1986). Blunters, on the other hand, often fail to adhere from the outset, since they underestimate their cancer risk. However, under conditions of relatively high threat (e.g., cancer diagnosis), even monitors can fail to adhere, since disease feedback generates sustained high levels of distress (Miller et al., 1994; Miller, Mischel, O'Leary, & Mills, 1996). Among women undergoing diagnostic follow-up, monitors reported greater intrusive ideation and consequent avoidant ideation than blunters; this distress, in turn, led to increased levels of denial, and mental and behavioral disengagement (Miller, Rodolitz, et al., 1996). The failure to modulate distress and intrusive ideation, accompanied by the use of avoidant coping strategies, can ultimately undermine adherence to appropriate health-protective behaviors and interfere with psychological adjustment (Schwartz et al., 1995).

Implications for the Tailoring of Health-Related Communications.

Research suggests that tailoring health-related communications to the individual's prototypic information processing style can facilitate adaptation in three main areas of health: 1) enhancing adjustment when undergoing diagnostic procedures and treatment regimens; 2) promoting adherence to recommended screening, diagnostic, and treatment regimens; and 3) facilitating informed decision making (Miller, 1995). First, with respect to interventions designed to enhance psychological adjustment to medical procedures, monitors (who are more attentive to threat), fare better when they receive detailed, reassuring information that prepares them for what they are about to experience; whereas, blunters prefer more minimal information (e.g., Miller & Mangan, 1983). For example, monitors undergoing follow-up diagnostic mammograms to assess abnormalities detected in prior screening benefited from receiving detailed preparatory information about the forthcoming diagnostic consultation (Sherman, 1999), in terms of anxiety, depression, and stress. Blunters, on the other hand, showed an increase in their negative affect when they received detailed preparatory information.

In another study, monitors demonstrated better psychological and behavioral adjustment to an aversive diagnostic procedure when they were helped to relax and raise their self-efficacy levels, compared to monitors who received no preparation (Gattuso, Litt, & Fitzgerald, 1992). High levels of detailed information and self-efficacy communications help the monitor to lower perceived vulnerability, reduce worry and uncertainty, and provide cues for planning, coping and managing anxiety. For blunters, detailed information may overwhelm them by forcing them to cognitively and affectively process information on which they would prefer not to focus (Miller, 1995).

With respect to interventions designed to promote adherence to healthcare recommendations, monitors generally experience emotional barriers (e.g., I'm too anxious to undergo regular Pap smears"), whereas blunters generally experience more encoding/expectancy barriers (e.g., I have no symptoms, therefore I must be fine"). Hence, health communication interventions need to be tailored to the distinctive cognitive-emotional profiles of these individuals. For example, among women undergoing diagnostic follow-up for an abnormal Pap smear, colposcopy, monitors fared better when they received detailed information and strategies for coping with distress; whereas blunters showed better adherence when merely told of the importance of follow-up appointments (Miller, Rodoletz, Buzaglo, Sherman, & Gray, 2001). In another study of women undergoing colposcopy, blunters reported greater adherence and knowledge when they received a preparatory message that targeted encoding barriers (i.e., which emphasized the costs of screening non-adherence). In contrast, monitors fared better when the same information was presented in a more neutral, reassuring fashion (Miler, Buzaglo, et al., 1999). Exposure to loss framing may serve to heighten a monitor's disease-specific intrusive ideation and sense of vulnerability, without promoting adaptive coping.

Finally, as advances in medical technology create more medical options for individuals, future studies need to investigate ways to tailor communications to promote effective decision making, especially in ambiguous contexts where no right or wrong answer exists, such as decisions about whether or not to undergo genetic testing or prophylactic surgery among health individuals. Interventions therefore need to be tailored to help the individuals assess and take account of their own information-processing styles before the decision is made. To accomplish this we have developed a Cognitive-Affective Processing (CAP) procedure, which is designed to help the individuals "pre-live" and anticipate their own cognitive and emotional reactions to different health care options. This type of pre-living can then be used as a more informed foundation on which to base health-related decisions and for coping with the psychosocial consequences of these decisions.

Conclusions

In sum, the cognitive-affective framework provides a theoretical basis from which to understand the dynamic processes involved in health-relevant information processing. With advances in medical technology, new challenges have arisen that require individuals to process large amounts of complex and unfamiliar health information and risk feedback, and to cope with high levels of uncertainty and ambiguity. For example, with the availability of genetic testing for inherited disease predisposition, individuals are confronted with numerous cognitive-affective challenges. These include decisions concerning whether or not to undergo genetic testing, how to cope with their testing decision, what to do with the test result information, to whom this information should be disclosed, and how to alter their behavior over time. Further, advances in medical treatment have led to an increase in disease survivorship and chronic illness which, in turn, have created unique challenges with respect to disease surveillance, treatment, and management. In this article, we have focused on two prototypic behavioral signatures that characterize individuals' response to health threats. Future research needs to explore the cognitive-affective profiles of monitors and blunders in a more fine-grained way, particularly in response to the maintenance of difficult future-oriented behaviors of the sort needed for long-term health-protective efforts (e.g., sustained mammography adherence, fitness) and information processing of ongoing risk feedback. This, in turn, will improve our ability to develop, and evaluate, theoretically guided informational and counseling interventions designed to enhance adjustment, promote adherence, and facilitate informed decision making by tailoring protocols to individual behavioral signatures. Finally, research needs to compare the impact of different modes of these evidence-based, tailored health-related communications delivered in more traditional forms (i.e., one-on-one counseling; telephone counseling; print communications) versus the use of more innovative novel technologies (e.g., interactive CD ROM; web-based communications).

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Presentation Abstract

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UGT1A6 GENETIC POLYMORPHISMS: IDENTIFICATION AND GENOTYPE/ PHENOTYPE ANALYSIS FROM HUMAN LIVER TISSUES. J.J. Zalatoris, PhD* and R.B. Raftogianis, PhD, Dept. Pharmacology, Fox Chase Cancer Center, Philadelphia, PA.

UDP glucuronosyltransferase (UGT) enzymes catalyze the glucuronidation of endogenous and exogenous molecules. UGT1A6 is expressed in several tissues, including the liver, and metabolizes small phenolic molecules. We set out to identify UGT1A6 alleles in a caucasian population and to determine the genotype/phenotype correlation of those alleles. We have identified three common single nucleotide polymorphisms (SNPs) in the first exon of UGT1A6 that alter encoded amino acids Ser7, Thr181 and Arg184. Different permutations of those three SNPs define four common alleles with frequencies of 0.69, 0.28, 0.022 and 0.011. SNP-specific RFLP assays were developed and applied to genotype DNA isolated from 43 random blood donors, 4 normal and 55 tumor liver tissues. Allele distribution followed the Hardy-Weinberg (HW) theorem in the random blood donors and normal liver tissue. Genotypes derived from liver tumors deviated from HW equilibrium such that the *1/*2 genotype was identified at a greater frequency than predicted. A spectrophotometric assay was developed to measure the rate of glucuronidation of naphthol and p-nitrophenol by UGT1A6. Microsomes prepared from the liver tissues were assayed to establish UGT1A6 phenotype. The most frequent genotypes, *1/*1 and *1/*2, were not associated with significantly different glucuronidation rates within liver tissues.

Identification of ovarian cancer-associated genes using a HOSE cell transformation model

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The diseases that are commonly referred to as ovarian cancer in the vast majority of cases develop from the malignant transformation of a single cell type, the surface epithelium. However, the biological mechanisms leading to transformation remain unclear. To identify genetic and biological determinants of ovarian cancer, we have developed an *in vitro* model for ovarian cancer. We have initiated primary human ovarian surface epithelial (HOSE) cell cultures and have derived HOSE cell lines that have undergone immortalization and spontaneous transformation *in vitro* and can form tumors *in vivo*. We have found that during immortalization, HOSE cells can use different pathways for telomere length maintenance. Maintenance of telomeric repeats is required for immortalization and is commonly associated with activity of telomerase. However, a number of tumors and tumor cell lines do not contain telomerase activity and circumvent the telomere length dependent limitation on cell division by a mechanism referred to as Alternative Lengthening of Telomeres (ALT). The mechanism(s) leading to ALT remains unknown. We have found that up to 30% of advanced stage ovarian adenocarcinomas lack telomerase activity. We have also observed that the majority of our HOSE cell cultures appear to use the ALT pathway for telomere maintenance, thereby providing an *in vitro* model to characterize the underlying basis of telomerase-dependent and independent ovarian tumorigenesis.

We have utilized Suppression Subtractive Hybridization (SSH) and cDNA microarray approaches to identify genes that are differentially expressed upon both immortalization and malignant transformation. One such gene, γ -synuclein, is a member of a family of small cytoplasmic proteins (i.e., α -, β -, γ -synuclein, and synoretin) that are predominantly expressed in neurons. The functions of the synucleins are not entirely understood, but they have been implicated in the pathogenesis of several neurodegenerative diseases. We have found that γ -synuclein, is expressed in the majority (>85%) of late-stage ovarian carcinomas, but it is not expressed in normal ovarian surface epithelium (Bruening, *et al.*, 2000). Therefore, we hypothesize that γ -synuclein may be a proto-oncogene and that abnormal expression of this protein in its oncogenic form may contribute to the progression and spread of ovarian cancer. In support of this hypothesis, we have observed that when γ -synuclein is exogenously expressed in cell lines derived from ovarian tumors, the cells become highly motile as observed by time-lapse photography and invasive as determined using a Boyden chamber assay. We further hypothesize that expression of γ -synuclein may be promoting this phenotype in part by modulating the Rho/Rac/CDC42 signal transduction pathway. Strikingly, levels of activated Rac (GTP-bound) are constitutively elevated in ovarian tumor cells that overexpress γ -synuclein.

Synuclein proteins also exhibit a weak homology to the 14-3-3 family of cytoplasmic chaperone proteins. The 14-3-3 family of proteins helps regulate many different signal transduction pathways, and is thought to act by directly binding to various protein kinases and bringing them into close proximity with substrate and regulatory proteins. We have recently demonstrated a novel interaction of γ -synuclein with a mitogen-activated kinase (MAPK), i.e., extracellular signal-regulated protein kinases (ERK1/2) and that overexpression of γ -synuclein leads to increased ERK activation. As activated ERK1/2 specifically localize to focal adhesions, and ERK1/2 has been shown to enhance migration, these results raise the possibility that γ -synuclein may enhance the metastatic potential of tumors through the activation of Rac within the Rho signaling pathway based on protein interactions at focal adhesions. Based on our results we hypothesize that γ -synuclein contributes to tumorigenesis by

promoting cell motility as a result of altering the Rho and ERK1/2 signaling pathways and that γ -synuclein may not only be a biomarker for ovarian cancer, but a therapeutic target.